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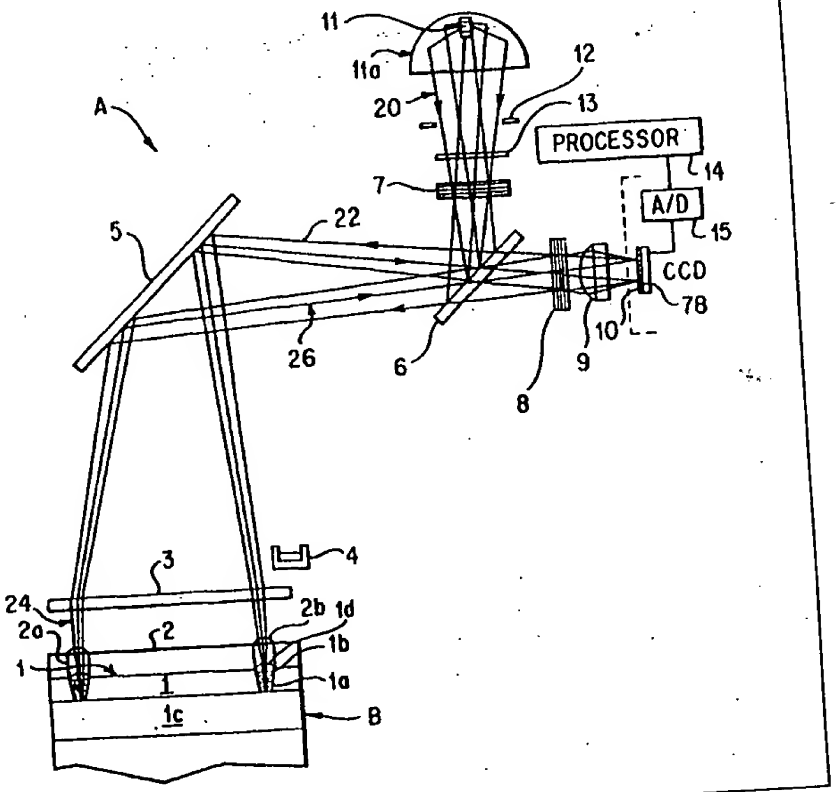
(54) Title: INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

(57) Abstract

An optical instrument monitors PCR replication of DNA in a reaction apparatus having a temperature cycled block with vials of reaction ingredients including dye that fluoresces in presence of double-stranded DNA. A beam splitter passes an excitation beam to the vials to fluoresce the dye. An emission beam from the dye is passed by the beam splitter to a CCD detector from which a processor computes DNA concentration. A reference strip with a plurality of reference emitters emit reference beams of different intensity, from which the processor selects an optimum emitter for compensating for drift. Exposure time is automatically adjusted for keeping within optimum dynamic ranges of the CCD and processor. A module of the beam splitter and associated optical filters is associated with selected dye, and is replaceable for different dyes.



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INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

This invention relates to biochemical analyses, and particularly to quantitative monitoring of DNA during a polymerase chain reaction (PCR) process.

BACKGROUND

Polymerase chain reaction (PCR) is a process for amplifying or multiplying quantities of double-stranded deoxyribonucleic acid (DNA). In a PCR apparatus, a thermal cycler block has one or more wells for holding vials containing a suspension of ingredients for a reaction to produce more DNA starting with "seed" samples of the DNA. The starting ingredients in an aqueous suspension, in addition to the a seed sample, include selected DNA primer strands, DNA elements, enzymes and other chemicals. The temperature of the block is cycled between a lower temperature extension phase of the PCR reaction at about 60°C, which is the phase where all of the DNA strands have recombined into double strands, and a high temperature denaturing phase at about 95°C, during which the DNA is denatured or split into single strands. Such a temperature program essentially doubles the DNA in each cycle, thus providing a method for replicating significant amounts of the DNA from a small starting quantity. The PCR process is taught, for example, in U.S. patent No. 4,683,202.

Quantitative measurements have been made on the DNA production during the PCR process, to provide measures of the starting amount and the amount produced. Measurements and computation techniques are taught in U.S. patent No. 5,766,889 (Atwood), as well as in an article "Kinetic PCR Analysis: Real-time Monitoring of DNA Amplification Reactions" by Russel Higuchi, et al., Bio/Technology vol. 11, pp. 1026-1030 (September 1993), and an article "Product Differentiation by Analysis of DNA Melting Curves during the Polymerase Chain Reaction" by Kirk M. Ririe, et al., Analytical Biochemistry vol. 245, pp. 154-160 (1997).

Prior measuring techniques have utilized microvolume fluorometers (spectrofluorometers) and a simple arrangement of a video camera with illumination lamps. Such apparatus utilize dyes that fluoresce in the presence of double-stranded DNA. These techniques and instruments are not particularly adapted to PCR apparatus for routine monitoring of the reaction. There also is a need for greater precision during the monitoring and measurements. Previous instruments that allow real time acquisition and analysis of PCR data have been very basic devices without the required dynamic range, do not have built-in calibration means, do not allow operation with sample well caps, or are very expensive.

An object of the present invention is to provide a novel optical instrument for quantitative monitoring of DNA replication in a PCR apparatus. Other objects are to provide such an instrument with improved dynamic range, automatic selection of exposure time to extend dynamic range, automatic adjustment for drift, simplified operation, relatively low cost, and easy changing of optics to accommodate different fluorescent dyes.

SUMMARY

The foregoing and other objects are achieved, at least in part, by an optical instrument as described herein for monitoring polymerase chain reaction replication of DNA. The replication is in a reaction apparatus that includes a thermal cycler block for holding at least one vial containing a suspension of ingredients for the reaction. The ingredients include a fluorescent dye that fluoresces proportionately in presence of DNA.

The instrument includes a light source, means for directing light beams, a light detector, and means for processing data signals. The light source emits a source beam having at least a primary excitation frequency that causes the dye to fluoresce at an emission frequency.. A first means is disposed to be receptive of the source beam to effect an excitation beam having the excitation frequency. A primary focusing means is disposed to focus the excitation beam into each suspension such that the primary dye emits an emission beam having the emission frequency and an intensity representative of concentration of DNA in each suspension. The focusing means is receptive of and passes

the emission beam. A second means is disposed to be receptive of the emission beam from the focusing means so as to further pass the emission beam at the emission frequency to another focusing means that focuses the emission beam onto a detector. The detector generates primary data signals representative of the emission beam and thereby a corresponding concentration of DNA in each vial. A processor is receptive of the primary data signals for computing and displaying the concentration of DNA.

In a preferred embodiment, the first means and the second means together comprise a beam splitter that is receptive of the source beam to effect the excitation beam, and receptive of the emission beam to pass the emission beam to the detector. The block is configured to hold a plurality of vials, and the focusing means comprises a corresponding plurality of vial lenses each disposed over a vial such that the emission beam comprises individual beams each associated with a vial. The focusing means may further comprise a field lens such as a Fresnel lens disposed cooperatively with the vial lenses to effect focusing of the excitation beam into each suspension, and to pass the individual beams to the second means (beam splitter). The detector preferably comprises an array of photoreceptors receptive of the individual beams to generate corresponding data signals such that the processing means computes concentration of DNA in each vial.

The instrument should also include an excitation filter between the light source and the beam splitter, and an emission filter between the beam splitter and the detector. The splitter and filters are associated with a selected primary dye in the suspension. In a further embodiment, a filter module contains the splitter and filters, and the module is removable from the housing for replacement with another module associated with another selected primary dye.

For a reference, a fluorescent reference member emits reference light in response to the excitation beam. The reference is disposed to be receptive of a portion of the excitation beam from the first means. A portion of the reference light is passed by the second means as a reference beam to the detector, so as to generate reference signals for utilization in the computing of the concentration of DNA. Preferably the reference member comprises

a plurality of reference emitters, each emitting a reference beam of different intensity in response to the excitation beam, to allow selection by the processor of a reference set having the highest data signals that are less than a predetermined maximum that is less than the saturation limit.

The detector is operatively connected to the processing means for the detector to integrate emission beam input over a preselected exposure time for generating each set of data signals, and the processing means or the detector or a combination thereof have a saturation limit for the data signals. In a further aspect of the invention, the processing means comprises adjustment means for automatically effecting adjustments in exposure time to maintain the primary data within a predetermined operating range for maintaining corresponding data signals less than the saturation limit, and means for correcting the primary data in proportion to the adjustments in exposure time. Preferably, the processor computes photoreceptor data from the data signals for each photoreceptor, and the adjustment means ascertains highest photoreceptor data, determines whether the highest photoreceptor data are less than, within or higher than the predetermined operating range and, based on such determination, the exposure time is increased, retained or reduced so as to effect a subsequent exposure time for maintaining subsequent photoreceptor data within the predetermined operating range.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of an optical train for an optical instrument according to the invention, associated with a polymerase chain reaction (PCR) reaction apparatus.

FIG. 2 is a perspective of the instrument of **FIG. 1** with a side panel removed.

FIG. 3 is an exploded perspective of a module shown in **FIG. 2**.

FIG. 4 is a perspective of a reference member in the optical train of **FIG. 1**.

FIG. 5 is a flow chart for computing DNA concentration from data obtained with the instrument of FIG. 1.

FIG. 6 is a flow chart for determining exposure time for data acquisition in operation of the instrument of FIG. 1 and for computations in the flow chart of FIG. 5.

FIG. 7 is a graph of extension phase data of fluorescence vs. cycles from operation of the instrument of FIG. 1 with a PCR apparatus.

FIG. 8 is a flow chart for computing secondary data for computations in the flow chart of FIG. 5.

FIG. 9 is a flow chart for computing ratios between the plurality of reference emitter segments of the reference member of FIG. 4.

DETAILED DESCRIPTION

An optical instrument A of the invention is utilized with or incorporated into a reaction apparatus B that replicates ("amplifies") selected portions of DNA by polymerase chain reaction ("PCR"). The reaction apparatus is conventional and should function without interference from the instrument which monitors the amount of DNA in real time during replication. Suitable reaction apparatus are described in U.S. patent Nos. 5,475,610 and 5,656,493.

The reaction apparatus (FIG. 1) is conventional and has two main components, namely a thermal cycler block 1 with wells 1a for holding at least one vial 1b containing a suspension of ingredients for the reaction, and a thermal cycle controller 1c for cycling the temperature of the block through a specified temperature program. The starting ingredients of the aqueous suspension of sample materials include a "seed" sample of DNA, selected DNA primer strands, DNA elements, enzymes and other chemicals. The block, typically aluminum, is heated and cooled in a prescribed cycle by electrical means,

liquid or air coolant, or a combination of these, or other means to achieve the cycling. The suspensions in the vials are thereby cycled between two temperature phases so as to effect the polymerase chain reaction. These phases are a lower temperature extension phase of the PCR reaction at about 60°C, which is the phase where all of the DNA strands have recombined into double strands, and a high temperature denaturing phase at about 95°C, during which the DNA is denatured or split into single strands.

For the present purpose the sample also contains a fluorescent dye that fluoresces proportionately and more strongly in the presence of double stranded DNA to which the dye binds, for example SYBR Green dye (available from Molecular Probes, Inc., Eugene, Oregon) that fluoresces in the presence of double stranded DNA. Another type of fluorescent dye labeled "probes", which are DNA-like structures with complimentary sequences to selected DNA strand portions, may also be used. Other dyes that have similar characteristics may be utilized. As used herein and in the claims, the term "marker dye" refers to the type that binds to double stranded DNA, or to the probe type, or to any other type of dye that attaches to DNA so as to fluoresce in proportion to the quantity of DNA. Samples may also contain an additional, passive dye (independent of the DNA) to serve as a reference as described below. Under incidence of light having a correct excitation frequency, generally a dye fluoresces to emit light at an emission frequency that is lower than that of the excitation light.

The vials typically are formed conically in a plastic unitary tray containing a plurality of vials, for example 96 in an array of 12 by 8. The tray preferably is removable from the block for preparations. A plastic unitary cover with caps **1d** for the vials may rest or attach over the vials to prevent contamination and evaporation loss. Other means may be used for this function, such as oil on the sample surface, in which case caps are not needed. If used, the caps are transparent to light utilized in the instrument, and may be convex facing upwardly.

The monitoring instrument is mounted over the block containing the vials. The instrument is removable or swings away for access to the vials. In the bottom of the instrument, a

platen 2 rests over the vial caps or, if none, directly over the vials. The platen, advantageously aluminum, has an array of holes 2a therethrough aligned with the vials, each hole having a diameter about the same as the vial top diameter. If there are caps, the platen should have its temperature maintained by a film heater or other means for heating the platen sufficiently to prevent condensation under the caps without interfering with DNA replication in the vials, for example holding the platen at slightly higher temperature than the highest sample temperature that the thermal cycler reaches.

Above each of the vials is a lens 2b positioned for its focal point to be approximately centered in the suspension in the vial. Above these lenses is a field lens 3 to provide a telecentric optical system. Advantageously the field lens is an aspherically corrected Fresnel lens for minimal distortion. A neutral density pattern (not shown) to correct nonuniformities in illumination and imaging may be mounted on or in proximity to the field lens, for example to attenuate light in the center of the image field. A folding optical mirror is optionally mounted at 45° for convenient packaging. This may be omitted, or other such folding optics may be used. Also the field lens, and/or the vial lenses, each may be comprised of two or more lenses that effect the required focusing, the word "lens" herein including such multiplicities.

A light source 11 for a source beam 20 of light is provided, for example a 100 watt halogen lamp. Preferably this is mounted at a focal distance of an ellipsoid reflector 11a which produces a relatively uniform pattern over the desired area. Also, advantageously, the reflector should be dichroic, i.e. substantially reflecting visible light and transmitting infrared light, to restrict infrared from the other optical components and from overheating the instrument. This is further aided by a heat reflecting mirror 13 in the optical path. A mechanical or electronic shutter 12 allows blockage of the light source for obtaining dark data. The type of light source is not critical, and other types may be used such as a projection lamp or a laser, with appropriate optical elements.

A beam splitter 6 is disposed to receive the source beam 20. In the present embodiment this is a dichroic reflector such that, positioned at 45°, it reflects light having an excitation

frequency that causes the marker dye to fluoresce at an emission frequency, and passes light having the emission frequency. Such a conventional optical device typically utilizes optical interference layers to provide the specific frequency response.

The beam splitter is positioned to reflect the source beam to the folding mirror. The source beam is reflected from the splitter as a excitation beam **22** having substantially the excitation frequency. The excitation beam is focused by the field lens **3** and then as separated beams **24** by the vial (well) lenses **2b** into the center of the vials. The marker dye is thereby caused to emit light at the emission frequency. This light is passed upwardly as an emission beam in the form of individual beams **26** that are reflected from the folding mirror **5** to the beam splitter **6** which passes the emission beam through to a detector **10**.

Together the vial lenses **2b** and the field lens **3** constitute a primary focusing means for focusing both the excitation beam and the emission beam. In an alternative aspect, the field lens may be omitted so that the focusing means consists only of the vial lenses **2b**. Alternatively, the vial lenses may be omitted so that the focusing means consists only of an objective lens in the field lens position to focus the individual emission beams on the detector.

Also, alternatively, the beam splitter **6** may pass the source beam as an excitation beam and reflect the emission beam, with appropriate rearrangement of the lamp and the detector. Moreover, other angles than 45° could be used if more suitable for the beam splitter, such as a more perpendicular reflection and pass through. More broadly, the beam splitter splits the optical paths for the excitation beam and the emission beam, and other variations that achieve this may be suitable. It is desirable to minimize source light reaching the detector, which the dichroic device helps achieve. A non-dichroic beam splitter may be used but would be less efficient as significant source light may reach the detector, or may be reflected or transmitted in the wrong direction and lost.

To further filter the source light, an excitation filter 7 is disposed between the light source 11 and the beam splitter 6. This passes light having the excitation frequency and substantially blocks light having the emission frequency. Similarly, an emission filter 8 is disposed between the beam splitter and the detector, in this case between the splitter and a detector lens 9 in front of the detector. This filter passes light having the emission frequency and substantially blocks light having the excitation frequency. Although a detector lens is preferred, a focusing reflector may be substituted for the detector lens. Such an emission focusing means (detector lens or reflector) may be located after (as shown) or before the beam splitter and on either side of the emission filter, and alternatively may be integrated into the primary focusing means. For example, the field lens may be an objective lens that focuses the emission beam onto the detector.

Suitable filters are conventional optical bandpass filters utilizing optical interference films, each having a bandpass at a frequency optimum either for excitation of the fluorescent dye or its emission. Each filter should have very high attenuation for the other (non-bandpass) frequency, in order to prevent "ghost" images from reflected and stray light. For SYBR Green dye, for example, the excitation filter bandpass should center around 485 nm wavelength, and the emission filter bandpass should center around 555 nm. The beam splitter should transition from reflection to transmission between these two, e.g. about 510 nm, so that light less than this wavelength is reflected and higher wavelength light is passed through.

More broadly, the excitation filter and the beam splitter together constitute a first means disposed to be receptive of the source beam to effect an excitation beam having the excitation frequency, and the emission filter and the beam splitter together constitute a second means disposed to be receptive of the emission beam from the focusing means so as to pass the emission beam at the emission frequency to the detector. Also, as mentioned above, the beam splitter alternatively may pass the source beam as an excitation beam and reflect the emission beam to the detector. In another aspect, the filters may be omitted, and the first means is represented by the beam splitter effecting the exitation

beam from the source beam, and the second means is represented by the beam splitter passing the emission beam to the detector.

In another arrangement, the beam splitter may be omitted, and the first means may constitute an excitation filter for the excitation frequency, the second means may constitute an emission filter for the emission frequency, with the light source and the detector being side by side so that the excitation and emission beams are on slightly different optical paths angularly. The source and detector need not actually be side by side with one or more folding mirrors. Thus any such arrangement for achieving the effects described herein should be deemed equivalent. However, use of the beam splitter is preferred so that the excitation and emission beams through the field lens will have the same optical path.

Advantageously the beam splitter **6**, the excitation filter **7** and the emission filter **8** are affixed in a module **30** (**FIG. 2**) that is associated with a selected primary dye for the suspension. The module is removable from the housing **32** of the instrument **A** for replacement with another module containing different beam splitter and filters associated with another selected primary dye. The instrument includes a lamp subhousing **33** and a camera subhousing **35**.

In an example (**FIG. 3**), each module includes a mounting block **34** with a flange **36** that is affixable to the housing with a single screw **38**. The beam splitter **6** is held at 45° in the block with a frame **40** and screws **42**. The emission filter **8** mounts (e.g. with glue) into the block. The excitation filter **7** mounts similarly into a mounting member **44** that is held by screws **46** to the block. With the module in place, the instrument is closed up with a side plate **47** that is screwed on. Positioning pins (not shown) ensure repeatable alignment. The replacement module may have the same mounting block and associated components, with the beam splitter and filters replaced.

The detector lens 9 (FIG. 1) is cooperative with the vial lenses 2b and the field lens 3 to focus the individual beams on the detector 10. The lens should be large aperture, low distortion and minimum vignetting.

The detector preferably is an array detector, for example a charge injection device (CID) or, preferably, a charge coupled device (CCD). A conventional video camera containing a CCD detector, the detector lens and associated electronics for the detector should be suitable, such as an Electrim model 1000L which has 751 active pixels horizontal and 242 (non-interlaced) active pixels vertical. This camera includes a circuit board that directly interfaces to a computer ISA bus. No framegrabber circuitry is required with this camera. Essentially any other digital imaging device or subsystem may be used or adapted that is capable of taking still or freeze-frame images for post processing in a computer.

A detector with a multiplicity of photoreceptors (pixels) 78 is preferable if there are a plurality of vials, to provide separate monitoring of each. Alternatively a scanning device may be used with a single photodetector, for example by scanning the folding mirror and using a small aperture to the detector. Also, a simple device such as a photomultipier may be used if there is only one vial. A CCD receives light for a selected integration period and, after analog/digital conversion, reads out digital signal data at a level accumulated in this period. The integration is effectively controlled by an electronic shutter, and a frame transfer circuit is desirable. Signal data are generated for each pixel, including those receiving the individual beams of emitted light from the vials.

The instrument preferably includes a fluorescent reference member 4 that emits reference light in response to the excitation beam. Advantageously the reference member is formed of a plurality of reference emitters, e.g. 6, each emitting a reference beam of different intensity in response to the excitation beam. The range of these intensities should approximate the range of intensities expected from the marker dye in the vials; for example each segment may be separated in brightness by about a factor of 2.5. The reference member is disposed to receive a portion of the excitation beam from the beam splitter. A good location is adjacent to the field lens, so that the optical paths associated

with the member approximate those of the vials. Most of the reference light passes back through the beam splitter as a reference beam to the detector. The detector pixels receive the emission beam to generate reference signals for utilization along with the data signals in the computing of the concentration of DNA.

Advantageously the reference member **4** (**FIG. 4**) comprises a plastic fluorescent strip **4a** and a neutral density filter **4b** mounted over the fluorescent strip, optionally with an air space **4h** between, such that a portion of the excitation beam and the reference beam are attenuated by the neutral density filter. The neutral density filter has a series of densities **4c** to effect the plurality of reference emitters (segments) each emitting a reference beam of different intensity. A heating strip **4d** and an aluminum strip **4g** to smooth the heating are mounted in a trough **4e** on the bottom thereof, and the fluorescent strip is mounted on the aluminum strip over the heating strip. To prevent heat loss, this assembly preferably is covered by a transparent plexiglass window (not shown, so as to display the varying density filter). To help maintain constant fluorescence, the heating strip is controlled to maintain the fluorescent strip at a constant temperature against the thermal cycles of the cycler block and other effects. This is done because most fluorescent materials change in fluorescence inversely with temperature.

The computer processor **14** (**FIG. 1**) may be a conventional PC. The computer programming is conventional such as with "C". Adaptations of the programming for the present invention will be readily recognized and achieved by those skilled in the art. The processor selectively processes signals from pixels receiving light from the vials and the reference emitters, ignoring surrounding light. The programming therefore advantageously includes masking to define the pixel regions of interest (ROI), e.g. as disclosed in copending provisional patent application serial No. 60/092,785 filed 07/14/98 of the present assignee. Mechanical alignment of the optics may be necessary to cooperatively focus the beams into the programmed regions of interest. The analog data signals are fed to the processor through an analog/digital (A/D) device **15** which, for the present purpose, is considered to be part of the processor. A saturation level is proscribed by either the detector or the A/D or, preferably, the CCD dynamic range is matched to the

A/D dynamic range. A suitable range is 8 bits of precision (256 levels), and the CCD amplifier offset is set so that the dark signal output of the CCD (with the shutter 12 closed) is within the A/D range. The processor instructs the detector with selected exposure time to maintain the output within the dynamic range.

In a typical operation, fluorescence data are taken from the plurality of vials (e.g. 96 regions of interest) and from the reference emitter segments, for each cycle in a DNA reaction replication sequence of thermal cycles, typically 40 to 50. Two data sets are taken (**FIG. 5**) for each thermal cycle during the extension phase of the PCR reaction at about 60°C, which is the phase where all of the DNA strands have recombined into double strands. One set is normal primary data 50 (along with reference data described below) and the other is dark signal data 51 with the mechanical shutter closed. Both digital data sets 50, 51 are converted by the A/D 15 from respective analog data signals 48, 49 from the detector. The dark are subtracted 55 from the normal, to yield dark-corrected data 57. In a simple procedure, the subtraction is pixel by pixel. Alternatively, total dark for each region of interest are subtracted from corresponding total fluorescence data. In another alternative, in order to increase effective dynamic range, it is advantageous to collect multiple exposures during each exposure period, e.g. 4 or 8 exposures. This is done by collecting multiple normal exposures and dark signal data for each pixel, subtracting each respective dark image from the normal data, then adding the subtracted data together to yield the primary data. This improves the statistical validity of the image data and increases its effective dynamic range.

Data are taken simultaneously from the reference strip which has, for example, 6 segments together with the 96 vials for a total of 102 regions of interest. Preferably the processing means provides for automatic adjustment of the exposure time to maintain the data signals within a predetermined operating range that is less than the saturation limit during the DNA replication sequence, for example 35% to 70% of saturation. Computations for DNA concentration include corrections in proportion to adjustments in exposure time (**FIG. 6**). Signal data 50, 51 from each exposure 52, 53 are obtained during a previously

determined exposure time 54 by totaling the pixel counts within each region of interest (ROI).

To provide the time adjustments, the highest signal data 56, which is data from one or more highest pixel readings, such as the three highest-reading contiguous pixels, is searched out 58 from the corresponding data signals 50. From a comparison 62 it is determined whether the highest signal data are less than, within or higher than the selected operating range 60. Based on such determination, the exposure time is adjusted 64, i.e. increased, retained or reduced, to obtain the subsequent exposure time 66. A reference time 68 (FIG. 5) also is selected which may be, for example, an initial time or a fixed standard time such as 1024 ms. The dark-corrected data 57 is time-corrected 69 to yield corrected primary data 71, dividing by ratio of actual exposure time to the reference time. The first several cycles may be out of range, and thereafter a useful fluorescence curve should be obtained (FIG. 7).

For the reference emitter, from the pixels receiving light from the reference strip 4 (FIGS. 1 and 4) reference data signals 73 are generated and converted by the A/D 15 to reference data 72. Selected reference data 74 from a specific reference segment 4c (FIG. 4) are selected 76 as that data having the highest signal strength that is less than a predetermined maximum 77 that, in turn, is less than the saturation limit, e.g. 70%. A next dimmer segment is also selected 75, and the selected reference data 74 include the data from that segment. The dark data 51 are subtracted 78 from the reference data 74, and the dark-corrected data 80 are adjusted 84 for exposure time 54 to yield adjusted reference data 82.

The data 82 includes dark corrected data 82' for the highest segment and dark corrected data 82" for the next dimmer segment (FIG. 9). The ratios of brightness between each segment are computed 89 and built up over the course of data collection. Each time data is collected, the ratio between the highest and next dimmer segment is calculated. As different optimum segments are selected on succeeding data collections, a table of ratios 85 is assembled. Alternatively, these rations may be collected and calculated in advance.

This adjusted reference data 82' (from data 82, **FIG. 5**) are utilized for computing normalized reference data 88 which are normalized 86 in real time as a ratio to reference data 90 from an initial or other selected previous cycle in the DNA replication (PCR) sequence by working back with the ratios 85. The normalized reference data are utilized on the corrected primary data 71 in a normalization computation 92 to provide drift normalized primary data 94 by dividing the primary data by the normalized reference data. This corrects for instrument drift during the monitoring. DNA concentration 96 may then be computed 98 from a stored calibration factors 99, determined by running standard known DNA concentrations to determine the slope and intercept of a line relating starting concentration to the starting cycle of the growth curve (**FIG. 7**) as taught in the aforementioned article by Higuchi and U.S. Patent No. 5,766,889. (Further normalization 118, 120 and baseline correction 122-130 are discussed below.)

Extension phase data for a typical PCR sequence would look like **FIG. 7**, plotted for each PCR cycle. If desired, the data may be corrected for dye bleaching or other sample chemical effects by normalizing to sample vials containing samples with the same dye and with DNA amplification prevented chemically.

The sample additionally may contain one or more types of dye molecules that serve as a "passive" reference having some fluorescence in the same wavelength range as the DNA binding dye. This reference dye is made up, for example, of a nucleic acid sequence labeled with Rhodamine and Fluorescein dye derivatives. A suitable reference is Rox dye from Perkin-Elmer Applied Biosystems. These passive dye molecules do not take part in the PCR reaction, so that their fluorescence is substantially without influence from DNA and remains constant during the reaction. This fluorescence can be used to normalize the fluorescence from the DNA binding dye with a standard concentration of passive dye included in the ingredients of at least one vial, preferably in every vial.

The source beam includes a secondary excitation frequency that causes the passive dye to fluoresce at a secondary frequency and thereby emit a secondary beam directed to the detector to generate corresponding secondary data signals. The processor is receptive of

the secondary data signals for computing secondary data representative of standard concentration. These data are used to normalize the primary data, so that the concentration of DNA is normalized to the standard concentration of passive dye after correcting computations of concentration of DNA in proportion to adjustments in exposure time, and in conjunction with the normalization for drift. Advantageously, and in the present example, the secondary excitation frequency is identical to the primary excitation frequency, and the passive dye fluoresces such that the emitted secondary beam is substantially at the emission frequency. The primary data signals are generated during each extension phase of cycling of the thermal cycler block when DNA is recombined and correspondingly primary dye emission is maximized. The secondary data signals are generated during each denature phase of cycling of the thermal cycler block when DNA is denatured and correspondingly primary dye emission is minimized. Thus data signals for the primary phase are substantially representative of DNA concentration, and data signals for the secondary phase are substantially representative of the standard concentration of passive dye.

The dark and normal data are taken for the vial samples and the reference strip, and the dark is subtracted from the normal fluorescence data. This dark and normal data set is taken during the extension phase of the PCR reaction at about 60°C, which is the phase where all of the DNA strands have recombined into double strands. During this phase, the fluorescence from the DNA binding dye is maximized, and the fluorescence from the passive reference molecules is superimposed but much smaller. A separate dark and normal data set is taken during the high temperature (about 95°C) denaturing phase, during which the DNA is denatured or split into single strands. During this phase, the fluorescence of the DNA binding dye is minimized, and almost non-existent, because the DNA is not double stranded and the fluorescence of the dyes used have a large decrease in fluorescence with increased temperature. Therefore the denaturing phase images substantially contain reference fluorescence from the passive reference molecules. The dark-corrected reference (denaturing) data set, after correction for measured temperature dependence, may be subtracted from the dark-corrected DNA binding dye data set, or may be deemed insignificant for the normal data set.

Alternatively, it may be desirable to image the passive reference dye labeled molecules by taking the additional images, for each PCR cycle, using a separate optical bandpass filter that rejects wavelengths emitted by the DNA binding dye while accepting wavelengths from the passive reference dye. This data would be functionally equivalent to the denature data.

Illustrating operation for the denature phase (**FIG. 8**), respective normal and dark data signals **102, 104** are obtained in the same manner as for the primary data, with normal exposure **52'** and closed shutter **53'**. Exposure time **106** may be the same as for an adjacent extension phase in the sequence, or determined from a previous denature phase run (as described with respect to **FIG. 7**), or may be a suitable time predetermined for all denature phases in the sequence. The A/D **15** converts the signals to secondary data **108** and dark data **110**. The dark is subtracted **55'** from the secondary to yield dark-corrected data **112** which is further corrected **69'** with a reference time **114** and the actual exposure time **106** to yield corrected secondary data **116.**

The extension cycle, drift normalized primary data **94** then are normalized **118** by dividing by the average of a selected number (e.g. 10) of cycles for the denature phase corrected secondary data **116** to produce further normalized fluorescence data or further normalized data **120**, which removes sample well to well non-uniformity effects. Cycle by cycle division may be used in place of an average. Alternatively the secondary data may be applied to the corrected primary data **71** before or after drift normalization. Baseline samples may be selected **122** and averaged **124** to produce baseline data **126**. The further normalized data **120** are then divided **128** by the baseline data to yield baseline corrected data **130**. These baseline samples are selected so as to be before the PCR growth exceeds the nearly horizontal base line portion of the curve in **FIG. 7**. Selected baseline cycles may be, for example, cycles 6 through 15. After further normalization **118**, the further normalized data **118** are used to compute **98** DNA concentration **96**.

The trend (e.g. least squares regression line) of these same baseline samples is subtracted from the normalized extension cycle data, to produce data that has a flat base line at zero. This data set may then be processed using established or other desired PCR methods to calculate the amount of starting copies of DNA. A simple procedure is to extrapolate for the inflection point at the transition from flat to rising. A more sophisticated procedure is described in the aforementioned U.S. patent No. 5,766,889.

The data may be used for various purposes, for example quantitative monitoring of the reaction or determination of replicated DNA concentration, or determination of the starting amount. The instrument also may be used (with or without normalizations and other corrections) simply to display whether replication is taking place during a sequence, or has taken place.

While the invention has been described above in detail with reference to specific embodiments, various changes and modifications which fall within the spirit of the invention and scope of the appended claims will become apparent to those skilled in this art. Therefore, the invention is intended only to be limited by the appended claims or their equivalents.

What is claimed is:

1. An optical instrument for monitoring polymerase chain reaction replication of DNA in a reaction apparatus that includes a thermal cycler block for holding at least one vial containing a suspension of ingredients for the reaction, the ingredients including a fluorescent primary dye that fluoresces proportionately in presence of DNA, the instrument comprising:

a light source for emitting a source beam having at least a primary excitation frequency that causes the primary dye to fluoresce at an emission frequency;

first means disposed to be receptive of the source beam to effect an excitation beam having the excitation frequency;

primary focusing means disposed to focus the excitation beam into each suspension such that the primary dye emits an emission beam having the emission frequency, the emission beam having an intensity representative of concentration of DNA in each suspension, the focusing means being receptive of and passing the emission beam;

second means disposed to be receptive of the emission beam from the focusing means so as to further pass the emission beam at the emission frequency;

emission focusing means for focusing the emission beam;

a detector disposed to be receptive of the emission beam from the second means and the emission focusing means such that the emission beam is focused onto the detector, the detector generating primary data signals representative of the emission beam and thereby a corresponding concentration of DNA in each vial; and

processing means receptive of the primary data signals for computing primary signal data and the corresponding concentration of DNA.

2. The instrument of claim 1 wherein the first means and the second means together comprise a beam splitter that is receptive of the source beam to effect the excitation beam, and receptive of the emission beam to pass the emission beam at the emission frequency to the detector.

3. The instrument of claim 2 wherein the beam splitter is disposed to reflect light having the excitation frequency and pass light having the emission frequency.

4. The instrument of claim 1 wherein the block is configured to hold a plurality of vials, the focusing means comprises a corresponding plurality of vial lenses each being disposed for positioning over a vial such that the emission beam comprises individual beams each associated with a vial, and the detector comprises an array of photoreceptors receptive of the individual beams to generate corresponding data signals such that the processing means computes concentration of DNA in each vial.

5. The instrument of claim 4 wherein the vials have transparent vial caps, the instrument further comprises a platen having holes therein aligned with the vial lenses so as to pass the individual beams and associated portions of the excitation beam therethrough, the platen being disposed for the holes to fit over the caps in contact therewith, and further comprises heating means for heating the platen sufficiently to prevent condensation under the caps without interfering with DNA replication in the vials.

6. The instrument of claim 4 wherein the focusing means further comprises a field lens disposed cooperatively with the vial lenses to effect focusing of the excitation beam into each suspension, and to pass the individual beams to the second means.

7. The instrument of claim 6 wherein the field lens is an aspherically corrected Fresnel lens.

8. The instrument of claim 6 wherein the emission focusing means comprises a detector lens disposed between the second means and the detector, the detector lens being cooperative with the vial lenses and the field lens to focus the individual beams on the detector.

9. The instrument of claim 8 further comprising a fluorescent reference emitter that emits reference light in response to the excitation beam, the reference emitter being disposed for the emission focusing means to focus at least a portion of the reference light as a reference

beam onto the detector, the detector being further receptive of the reference beam to generate a reference signal, and the processing means comprises means receptive of the reference signal for computing reference data, and means for normalizing the primary data with the reference data for a chosen point in the reaction replication of DNA, thereby correcting for instrument drift during the monitoring.

10. The instrument of claim 9 wherein the reference member comprises a plurality of reference emitters each emitting a reference beam of different intensity in response to the excitation beam, the reference emitters being disposed for the emission focusing means to focus each reference beam onto the detector, the detector being further receptive of each reference beam to generate a set of reference signals for each reference emitter, and the processing means comprises means receptive of the reference signals for computing corresponding sets of reference data, means for selecting from the sets the selected reference data that has the highest signal data less than a predetermined maximum, the selected reference data being used for normalizing the primary data.

11. The instrument of claim 1 wherein the first means further comprises an excitation filter, the second means further comprises an emission filter, and the first means and the second means together comprise a beam splitter, the excitation filter being disposed between the light source and the beam splitter, the emission filter being disposed between the beam splitter and the detector, the excitation filter passing light having the excitation frequency and substantially blocking light having the emission frequency, and the emission filter passing light having the emission frequency and substantially blocking light having

the excitation frequency, the excitation filter and the beam splitter being cooperatively receptive of the source beam to effect the excitation beam, and the emission filter and the beam splitter being cooperatively receptive of the emission beam to pass the emission beam having the emission frequency to the detector.

12. The instrument of claim 11 further comprising a housing containing the light source, the detector, the focusing means and a filter module, wherein the beam splitter, the excitation filter and the emission filter are affixed in the module and are associated with a selected primary dye for the suspension, and the module is removable from the housing for replacement with another module associated with another selected primary dye.

13. The instrument of claim 1 wherein the light source comprises a halogen lamp and an ellipsoid reflector disposed proximate to the lamp oppositely from the first means, the lamp being disposed at a focal distance from the ellipsoid reflector to effect the source beam with light reflected from the ellipsoid reflector, and the ellipsoid reflector substantially reflecting visible light and transmitting infrared light.

14. The instrument of claim 1 further comprising a fluorescent reference emitter that emits reference light in response to the excitation beam, the reference emitter being disposed for the emission focusing means to focus at least a portion of the reference light as a reference beam onto the detector, the detector being further receptive of the reference beam to generate a reference signal, and the processing means comprises means receptive of the reference signal for computing reference data, and means for normalizing the

primary data with the reference data for a chosen point in the reaction replication of DNA, thereby correcting for instrument drift during the monitoring.

15. The instrument of claim 14 wherein the reference member comprises a plurality of reference emitters each emitting a reference beam of different intensity in response to the excitation beam, the reference emitters being disposed for the emission focusing means to focus each reference beam onto the detector, the detector being further receptive of each reference beam to generate a set of reference signals for each reference emitter, and the processing means comprises means receptive of the reference signals for computing corresponding sets of reference data, means for selecting from the sets the selected reference data that has the highest signal data less than a predetermined maximum, the selected reference data being used for normalizing the primary data.

16. The instrument of claim 15 wherein the reference member comprises a plastic fluorescent strip and a neutral density filter mounted on the fluorescent strip such that the reference beam and a portion of the excitation beam are attenuated by the neutral density filter, the neutral density filter having a series of densities to effect the plurality of reference emitters each emitting a reference beam.

17. The instrument of claim 16 further comprising temperature means for maintaining the reference member at substantially constant temperature.

18. The instrument of claim 17 wherein the temperature means comprises a heating strip mounted under the fluorescent strip, and means for controllably heating the heating strip.

19. The instrument of claim 1 further comprising a plurality of fluorescent reference emitters each emitting a reference beam of different intensity in response to the excitation beam, the reference emitters being disposed for the emission focusing means to focus each reference beam onto the detector, the detector being further receptive of each reference beam to generate a set of reference signals for each reference emitter, and the processing means comprises means receptive of the reference signals for computing corresponding sets of reference data, means for selecting from the sets the selected reference data that has the highest signal data less than a predetermined maximum, and means for normalizing the primary data with corresponding selected reference data for a chosen point in the reaction replication of DNA, thereby correcting for instrument drift during the monitoring.

20. The instrument of claim 1 wherein sets of data signals are generated sequentially in a replication sequence, the processing means or the detector or a combination thereof have a saturation limit for the data signals in each set, the detector is operatively connected to the processing means for the detector to integrate emission beam input over a preselected exposure time for generating each set of data signals, and the processing means comprises adjustment means for automatically effecting adjustments in exposure time to maintain the primary data within a predetermined operating range for maintaining corresponding data signals less than the saturation limit, and means for correcting the primary data in proportion to the adjustments in exposure time.

21. The instrument of claim 20 wherein the detector comprises an array of photoreceptors receptive of the emission beam for generating corresponding data signals in an associated exposure time, the predetermined operating range is for each photoreceptor, and the processing means further comprises means for computing photoreceptor data from the data signals for each photoreceptor, and the adjustment means comprises means for ascertaining highest photoreceptor data, means for determination of whether the highest photoreceptor data are less than, within or higher than the predetermined operating range, and means based on such determination for respectively increasing, retaining or reducing the exposure time so as to effect a subsequent exposure time for maintaining subsequent photoreceptor data within the predetermined operating range.

22. The instrument of claim 1 further comprising a plurality of fluorescent reference emitters each emitting a reference beam of different intensity in response to the excitation beam, the reference emitters being disposed for the emission focusing means to focus each reference beam onto the detector, the detector being further receptive of each reference beam to generate a set of reference signals for each reference emitter, and the processing means comprises means receptive of the reference signals for computing corresponding sets of reference data, means for selecting from the sets the selected reference data that has the highest signal data less than a predetermined maximum, and means for normalizing the primary data with corresponding selected reference data for a chosen point in the reaction replication of DNA, thereby correcting for instrument drift during the monitoring.

23. The instrument of claim 20 wherein the ingredients for at a vial further include a standard concentration of fluorescent passive dye that fluoresces substantially without influence from DNA, the source beam includes a secondary excitation frequency that causes the passive dye to fluoresce at a secondary emission frequency and thereby emit a secondary emission beam passed by the second means and focused onto the detector to generate corresponding secondary data signals, the processing means further comprises means receptive of the secondary data signals for computing secondary data, and means for dye normalizing the primary data, whereby the computed concentration of DNA is normalized to the standard concentration of passive dye.

24. The instrument of claim 23 wherein the secondary excitation frequency is identical to the primary excitation frequency, the passive dye fluoresces such that the secondary beam is substantially at the emission frequency, the primary data signals are generated during an extension phase of cycling of the thermal cycler block when DNA is recombined and correspondingly primary dye emission is maximized, and the secondary data signals are generated during a denature phase of cycling of the thermal cycler block when DNA is denatured and correspondingly primary dye emission is minimized, whereby data signals for the extension phase are substantially representative of DNA concentration and data signals for the denature phase are substantially representative of the standard concentration of passive dye.

25. The instrument of claim 1 wherein the ingredients for a vial further include a standard concentration of fluorescent passive dye that fluoresces substantially without influence

from DNA, the source beam includes a secondary excitation frequency that causes the passive dye to fluoresce at a secondary emission frequency and thereby emit a secondary emission beam passed by the second means and focused onto the detector to generate corresponding secondary data signals, the processing means comprises means receptive of the secondary data signals for computing secondary data, and means for dye normalizing the primary data, whereby the computed concentration of DNA is normalized to the standard concentration of passive dye.

26. The instrument of claim 25 wherein the secondary excitation frequency is identical to the primary excitation frequency, the passive dye fluoresces such that the secondary beam is substantially at the emission frequency, the primary data signals are generated during an extension phase of cycling of the thermal cycler block when DNA is recombined and correspondingly primary dye emission is maximized, and the secondary data signals are generated during a denature phase of cycling of the thermal cycler block when DNA is denatured and correspondingly primary dye emission is minimized, whereby data signals for the extension phase are substantially representative of DNA concentration and data signals for the denature phase are substantially representative of the standard concentration of passive dye.

27. A system for replication of DNA and monitoring thereof, comprising a reaction apparatus for polymerase chain reaction replication of DNA, and an optical instrument for monitoring presence of DNA during such replication, wherein the apparatus comprises a thermal cycler block for holding at least one vial containing a suspension of ingredients for

the reaction, the ingredients including a fluorescent dye that fluoresces proportionately in presence of DNA, and further comprises means for thermal cycling the block and thereby the suspension so as to effect the polymerase chain reaction; wherein the instrument comprises:

a light source for emitting a source beam having at least an excitation frequency that causes the dye to fluoresce at an emission frequency;

first means disposed to be receptive of the source beam to effect an excitation beam having the excitation frequency and to pass the excitation beam to a focusing means, the focusing means being for focusing the excitation beam into each suspension such that the dye emits an emission beam having the emission frequency, and for passing the emission beam to a second means, the second means being for further passing the emission beam to a detector, the detector being disposed to be receptive of the emission beam from the second means so as to generate data signals representative of the emission beam and thereby concentration of DNA; and

processing means receptive of the data signals for computing and displaying the concentration of DNA.

28. The system of claim 27 wherein the first means and the second means together comprise a beam splitter receptive of the source beam to effect the excitation beam, and receptive of the emission beam to pass the emission beam to the detector.

29. A filter module for an optical instrument that monitors polymerase chain reaction replication of DNA in a reaction apparatus that includes a thermal cycler block for holding at least one vial containing a suspension of ingredients for the reaction, the ingredients including a fluorescent dye that fluoresces proportionately in presence of DNA, the instrument including a housing, a light source disposed in the housing for emitting a source beam having at least an excitation frequency that causes the dye to fluoresce at an emission frequency, focusing means disposed in the housing for focusing an excitation beam having the excitation frequency into each suspension such that the dye emits an emission beam having the emission frequency, a detector disposed in the housing to be receptive of the emission beam so as to generate data signals representative of the emission beam and thereby concentration of DNA, and processing means receptive of the data signals for computing and displaying the concentration of DNA; wherein the module comprises:

a support frame, the instrument being receptive of the support frame into the instrument;

a beam splitter affixed in the support frame so that, with the module inserted, the beam splitter is receptive of the source beam so as to effect the excitation beam, and receptive of the emission beam to pass the emission beam to the detector;

an excitation filter that passes light having the excitation frequency and substantially blocks light having the emission frequency, the excitation filter being affixed in the support

frame so that, with the module inserted, the excitation filter is disposed between the light source and the beam splitter; and

an emission filter that passes light having the emission frequency and substantially blocks light having the excitation frequency, the emission filter being affixed in the support frame so that, with the module inserted, the emission filter is disposed between the detector and the beam splitter;

the beam splitter, the excitation filter and the emission filter, and thereby the module, being associated with a selected dye for the suspension, and the module being removable from the housing for replacement with another module associated with another selected dye.

30. The module of claim 29 wherein the beam splitter reflects light having an excitation frequency, and passes light having the emission frequency.

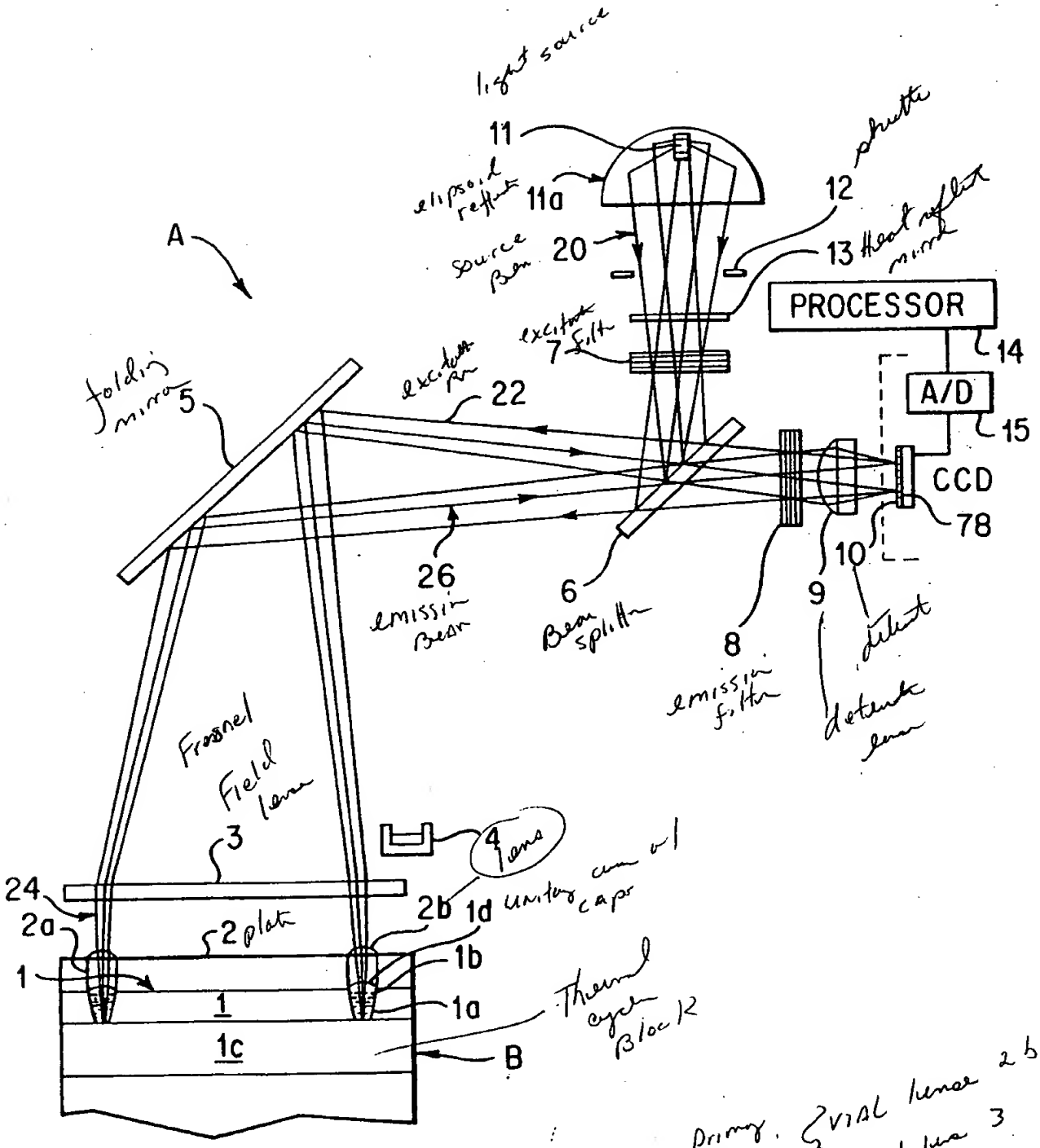

FIG. 1

Primary focusing means { VIAL lenses 2b
Field lens 3

1st means to be reception of the same { excitation filter and/or Beam splitter

2nd means { emission filter and/or beam splitter

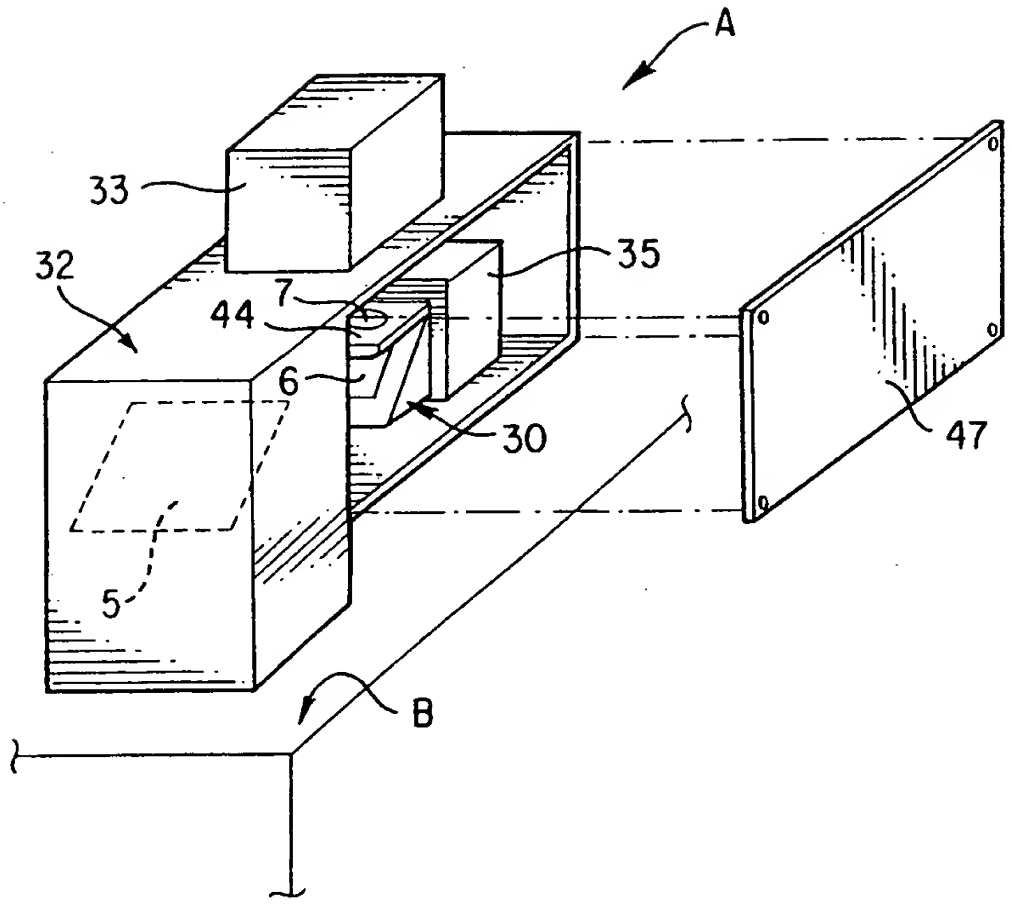

FIG. 2

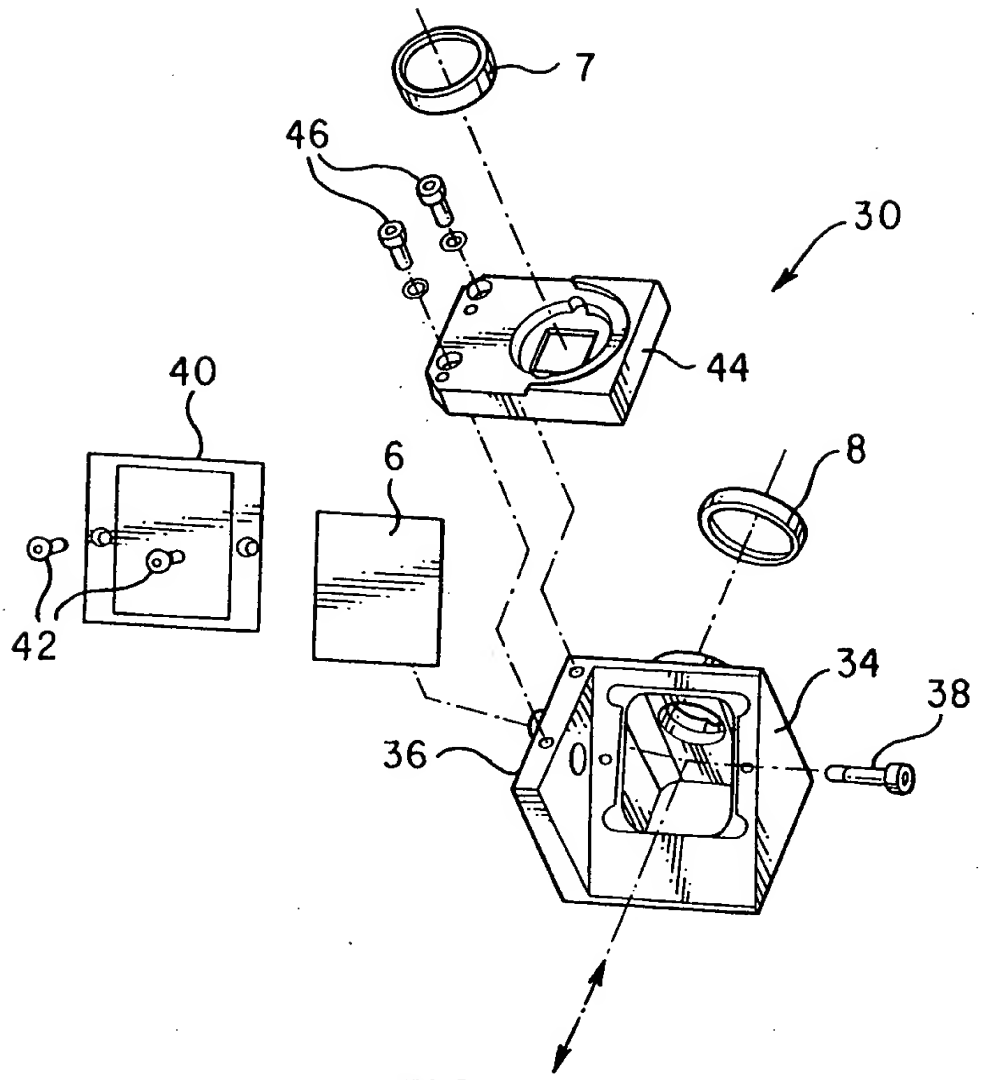
7
46
30
44
40
6
8
42
34
38
36

FIG. 3

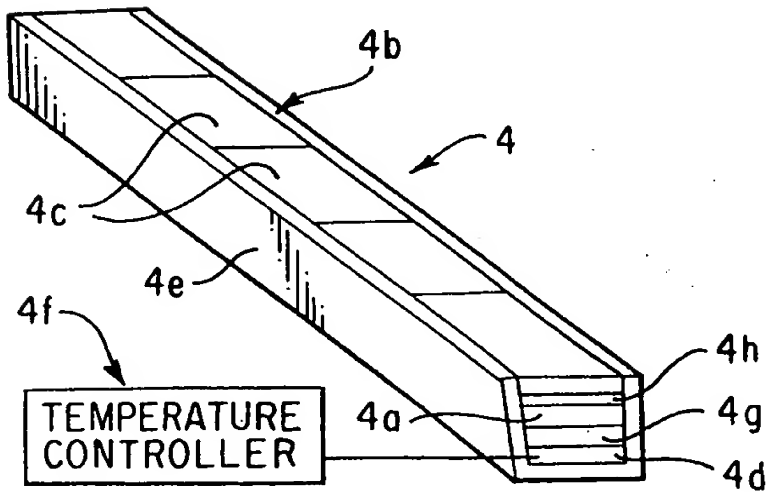
4b
4
4c
4e
4f
TEMPERATURE CONTROLLER
4h
4a
4g
4d

FIG. 4

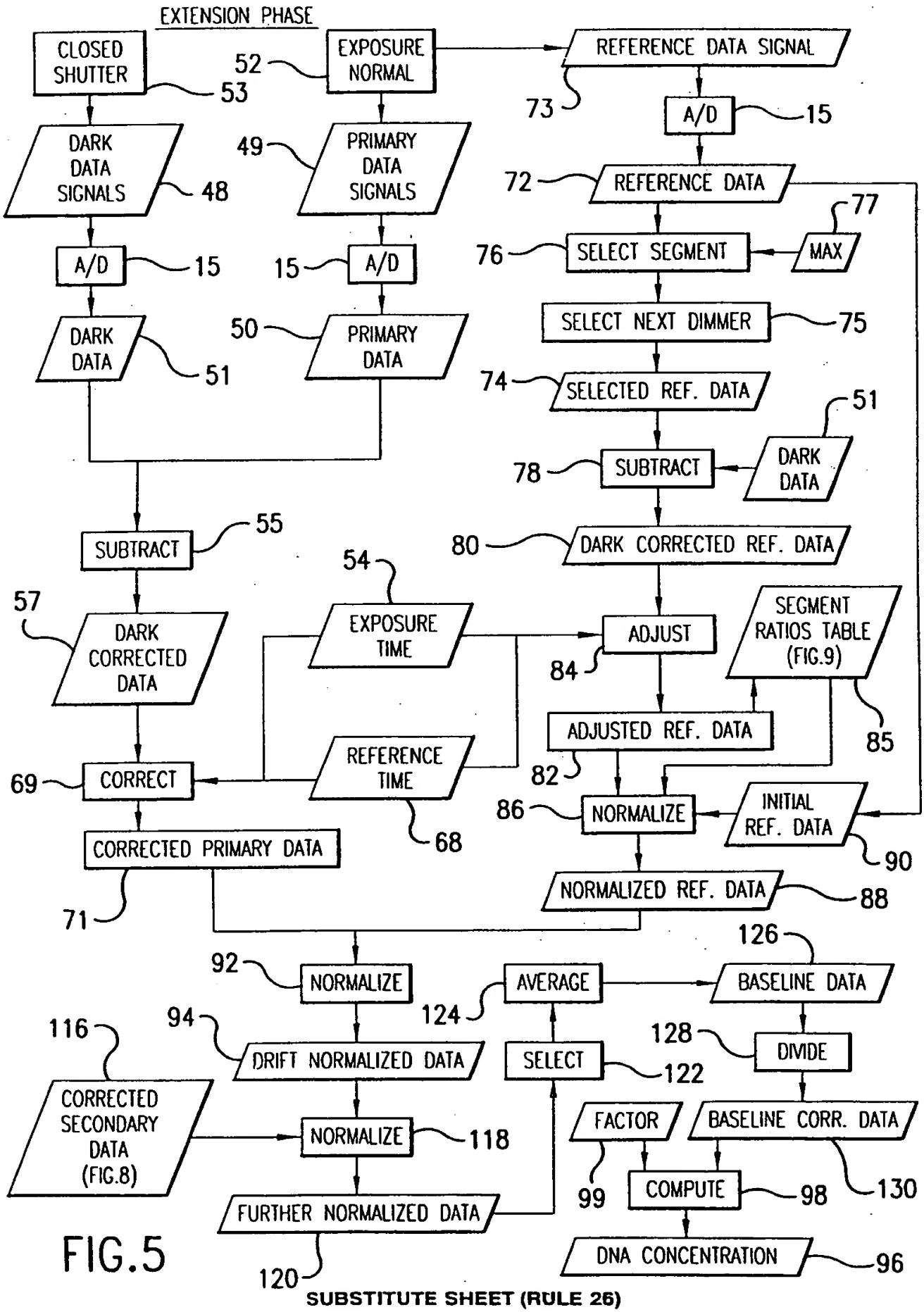
EXTENSION PHASE
CLOSED SHUTTER
53
EXPOSURE NORMAL
52
REFERENCE DATA SIGNAL
73
A/D
15
DARK DATA SIGNALS
48
49
PRIMARY DATA SIGNALS
72
REFERENCE DATA
77
A/D
15
15
A/D
76
SELECT SEGMENT
MAX
DARK DATA
51
50
PRIMARY DATA
SELECT NEXT DIMMER
75
74
SELECTED REF. DATA
51
78
SUBTRACT
DARK DATA
SUBTRACT
55
80
DARK CORRECTED REF. DATA
54
57
DARK CORRECTED DATA
EXPOSURE TIME
ADJUST
84
SEGMENT RATIOS TABLE (FIG.9)
ADJUSTED REF. DATA
85
82
69
CORRECT
REFERENCE TIME
68
86
NORMALIZE
INITIAL REF. DATA
90
CORRECTED PRIMARY DATA
71
NORMALIZED REF. DATA
88
126
92
NORMALIZE
AVERAGE
BASELINE DATA
124
116
94
DRIFT NORMALIZED DATA
SELECT
122
128
DIVIDE
CORRECTED SECONDARY DATA (FIG.8)
NORMALIZE
118
FACTOR
BASELINE CORR. DATA
130
99
COMPUTE
98
FURTHER NORMALIZED DATA
120
DNA CONCENTRATION
96

FIG.5

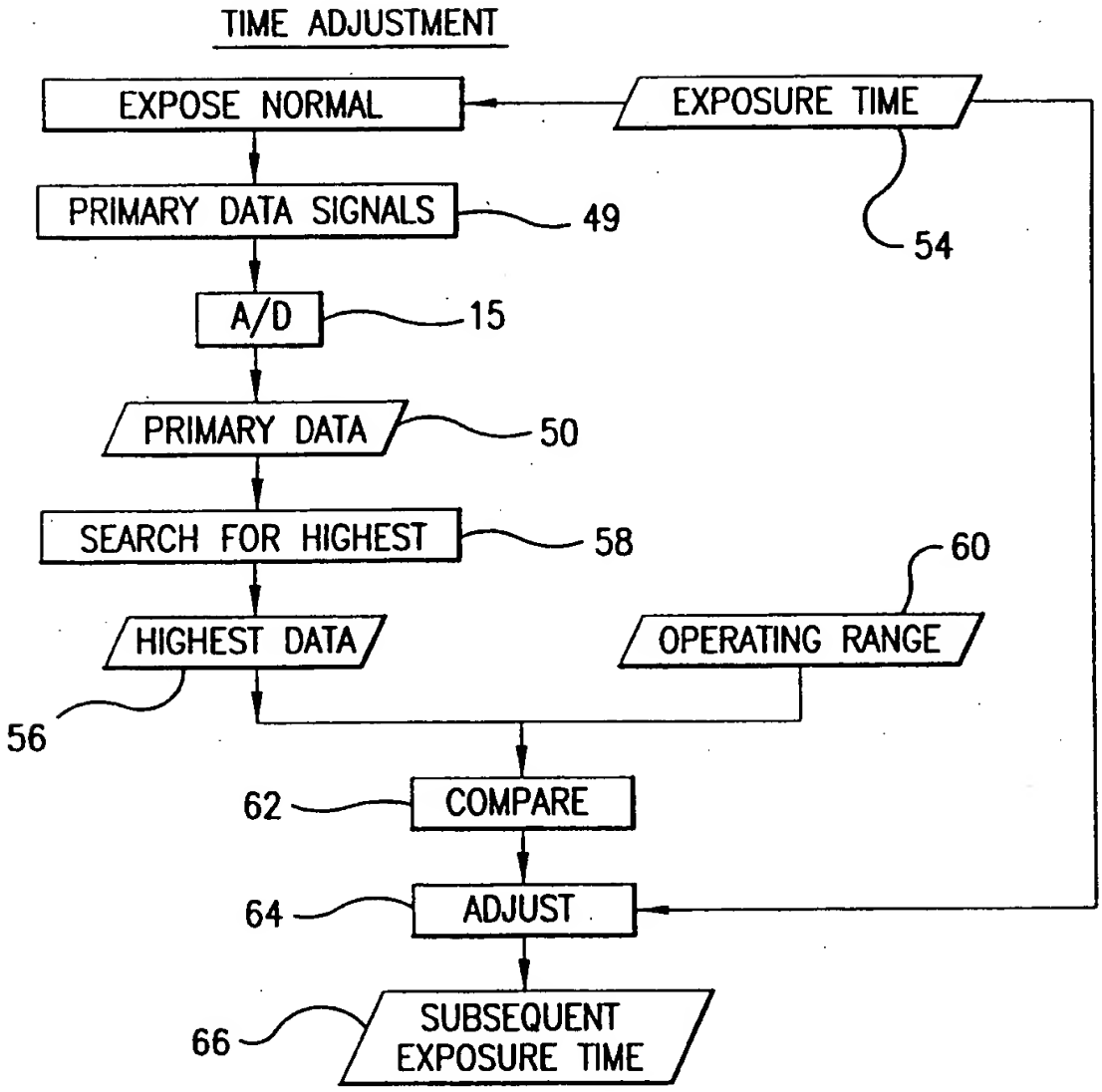
TIME ADJUSTMENT
EXPOSE NORMAL
EXPOSURE TIME
PRIMARY DATA SIGNALS
49
54
A/D
15
PRIMARY DATA
50
SEARCH FOR HIGHEST
58
60
HIGHEST DATA
OPERATING RANGE
56
62
COMPARE
64
ADJUST
66
SUBSEQUENT EXPOSURE TIME

FIG.6

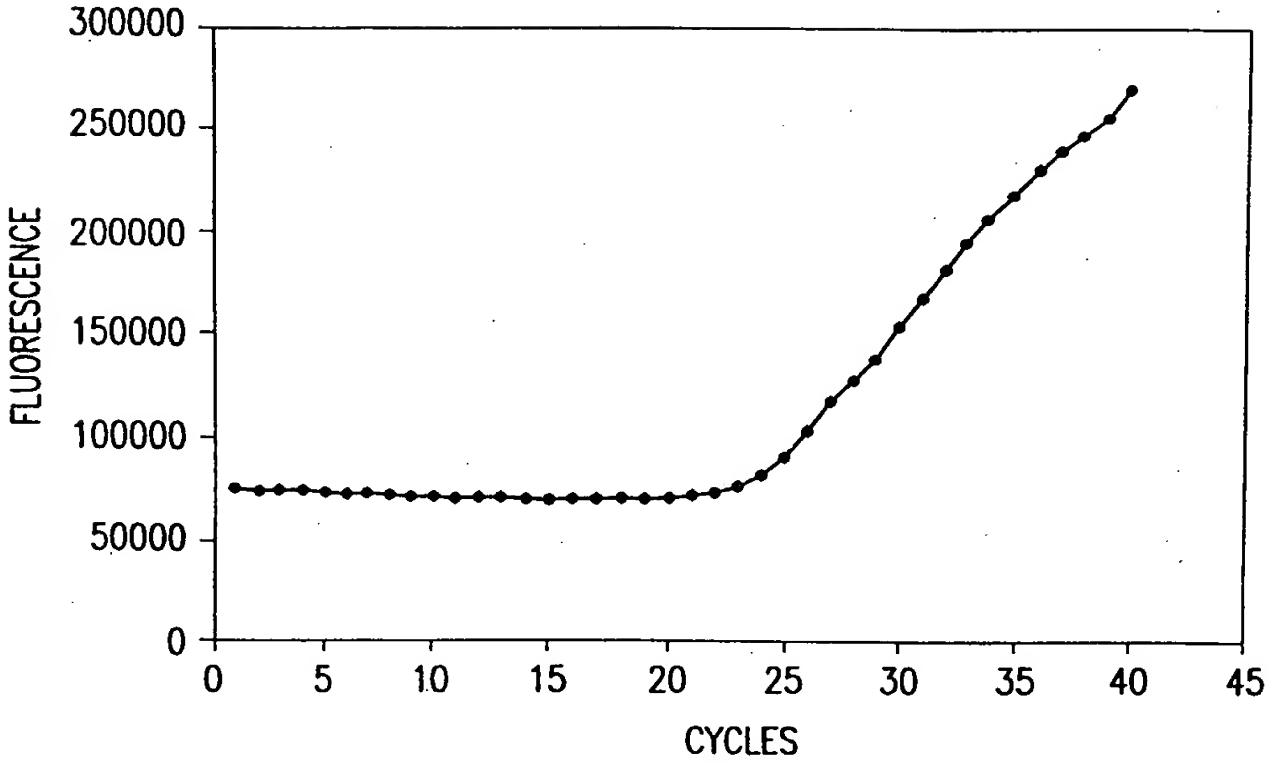
300000
250000
200000
150000
100000
50000
0
FLUORESCENCE
0
5
10
15
20
25
30
35
40
45
CYCLES

FIG.7

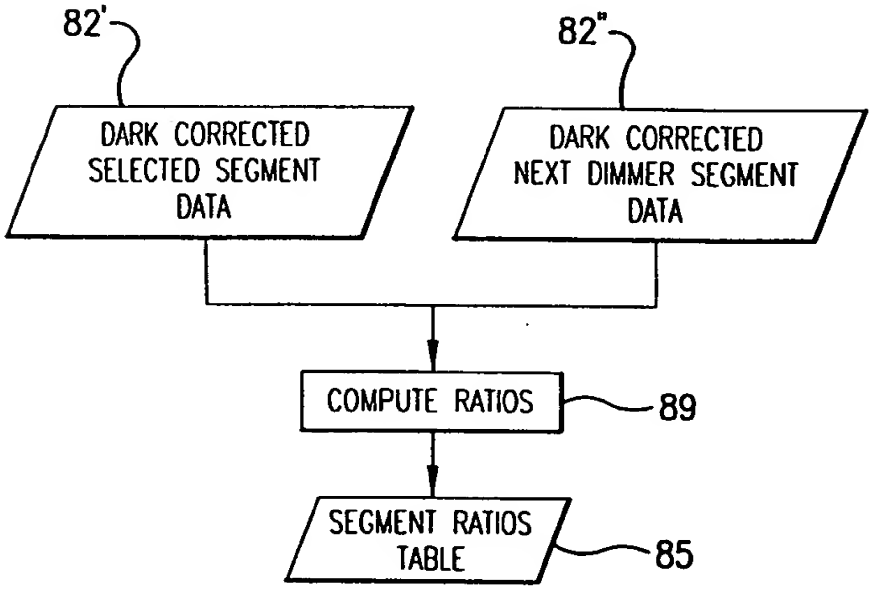
82'
82"
DARK CORRECTED SELECTED SEGMENT DATA
DARK CORRECTED NEXT DIMMER SEGMENT DATA
COMPUTE RATIOS
89
SEGMENT RATIOS TABLE
85

FIG.9

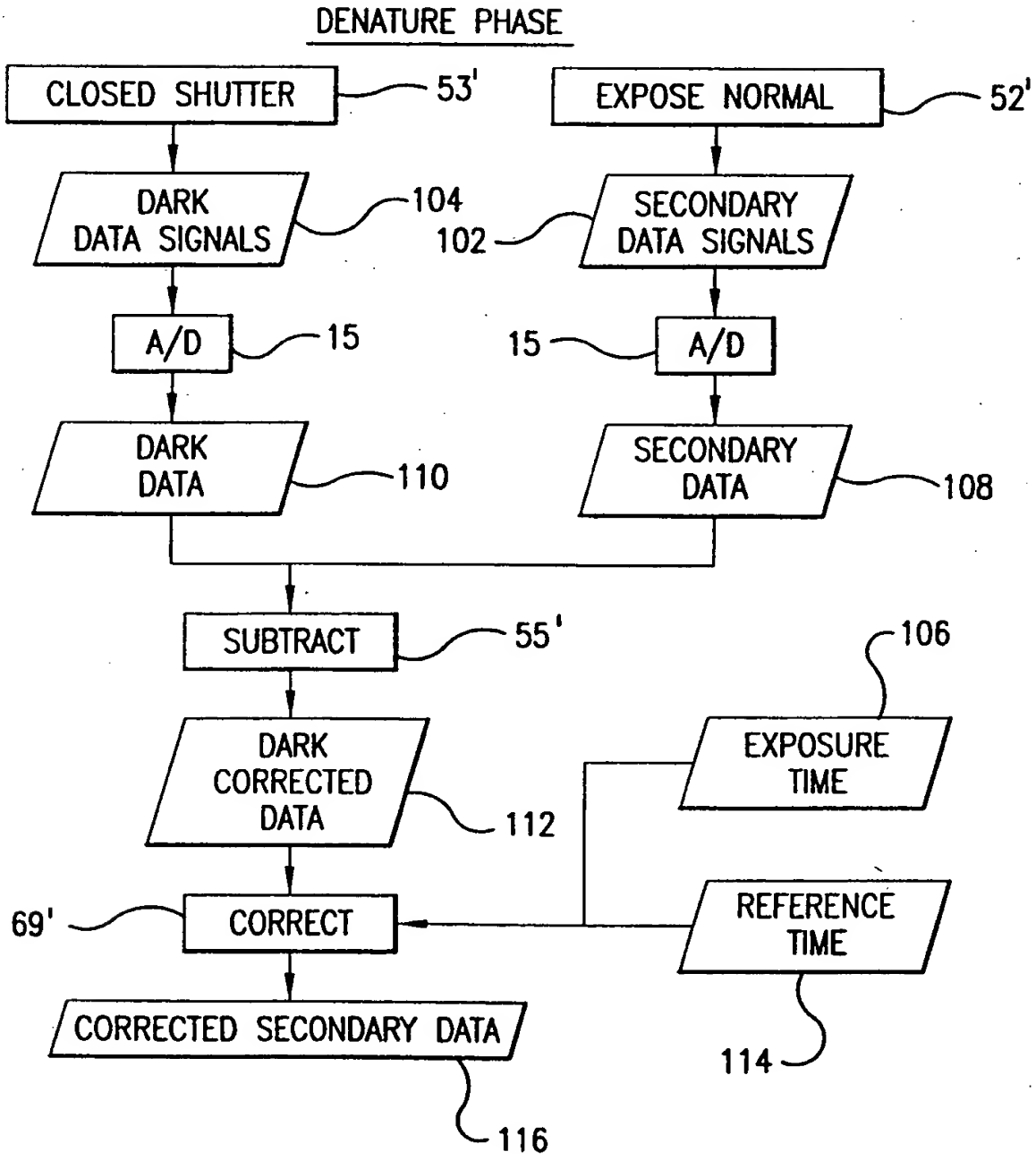
DENATURE PHASE
CLOSED SHUTTER
53'
EXPOSE NORMAL
52'
DARK DATA SIGNALS
104
SECONDARY DATA SIGNALS
102
A/D
15
15
A/D
DARK DATA
110
SECONDARY DATA
108
SUBTRACT
55'
106
DARK CORRECTED DATA
112
EXPOSURE TIME
69'
CORRECT
REFERENCE TIME
CORRECTED SECONDARY DATA
114
116

FIG.8

INTERNATIONAL SEARCH REPORT

[redacted] International Application No
[redacted]/US 99/11088

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/25 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 736 333 A (MCBRIDE LINCOLN J ET AL) 7 April 1998 (1998-04-07) figures 1,2 ---	1-30
Y	EP 0 640 828 A (HOFFMANN LA ROCHE) 1 March 1995 (1995-03-01) figure 15 ---	1-30
A	WO 97 23649 A (BIORAD LAB INC) 3 July 1997 (1997-07-03) figure 1 ---	1-30
A	WO 97 46707 A (RASMUSSEN RANDY P ;UNIV UTAH RES FOUND (US); RIRIE KIRK M (US); WI) 11 December 1997 (1997-12-11) figure 11 --- -/--	1-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 1999	15/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Mason, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTE[redacted]TIONAL SEARCH REPORT

[redacted]tional Application No
PC./US 99/11088

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 622 455 A (BECTON DICKINSON CO) 2 November 1994 (1994-11-02) figures 1,5	1-30

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[illegible]on on patent family members

[illegible]ational Application No
[illegible]/US 99/11088

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5736333 A	07-04-1998	AU 3230297 A	05-01-1998
		CA 2256457 A	11-12-1997
		EP 0904411 A	31-03-1999
		WO 9746708 A	11-12-1997
EP 0640828 A	01-03-1995	AU 681682 B	04-09-1997
		AU 7141494 A	09-03-1995
		BR 9403338 A	11-04-1995
		CA 2129787 A	28-02-1995
		CN 1107892 A	06-09-1995
		CZ 9402078 A	15-11-1995
		FI 943936 A	28-02-1995
		HU 71622 A	29-01-1996
		IL 110732 A	15-07-1998
		JP 7163397 A	27-06-1995
		NO 943166 A	28-02-1995
		NZ 264310 A	25-03-1998
		PL 304805 A	06-03-1995
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		ZA 9406330 A	28-02-1995
WO 9723649 A	03-07-1997	US 5792610 A	11-08-1998
		AU 1423597 A	17-07-1997
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		EP 0874912 A	04-11-1998
		EP 0879297 A	25-11-1998
		WO 9723648 A	03-07-1997
		US 5759781 A	02-06-1998
WO 9746707 A	11-12-1997	AU 3154797 A	05-01-1998
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		WO 9746712 A	11-12-1997
		WO 9746714 A	11-12-1997
EP 0622455 A	02-11-1994	US 5371016 A	06-12-1994
		AU 668278 B	26-04-1996
		AU 5919894 A	27-10-1994
		BR 9401570 A	22-11-1994
		CA 2120993 A	27-10-1994
		JP 2738500 B	08-04-1998
		JP 6319524 A	22-11-1994

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

AKER, David
PERMAN & GREEN, LLP
425 Post Road
Fairfield
Connecticut 06430-6232
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	16.08.2000
Applicant's or agent's file reference 715-009111WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/11088	17/05/1999	16/05/1998

Applicant
THE PERKIN-EIMER CORPORATION et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Conner, M Tel.+49 89 2399-2241

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
715-009111WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/11088	17/05/1999	16/05/1998
International Patent Classification (IPC) or national classification and IPC G01N21/25		
Applicant THE PERKIN-EIMER CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/12/1999	16.08.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mason, W Telephone No. +49 89 2399 2623

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/11088

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-18 as originally filed

Claims, No.:

1-30 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26,28-30
	No:	Claims	27
Inventive step (IS)	Yes:	Claims	16-18
	No:	Claims	1-15,19-26,28-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations

see separate sheet

WITH RESPECT TO POINT V

1. The following documents are referred to in this report:

D1=US5736333; D2=EP0640828; D3=US5792610; D4=WO9746707

The present application relates to optical apparatus for monitoring polymerase chain reactions for replication of DNA held in vials of a thermal cycler block. The apparatus comprises arrangement of light sources, filters, detectors, beam splitters, and lenses in particular arrangements.

2. PRELIMINARY

In as much as any of the claims are directed to apparatus which does not contain features relating to signals representative of the DNA sample, such claimed apparatus is not limited by the form of the sample itself (DNA replication fluids) and prior art disclosing the same apparatus features for samples of a different type must still be considered relevant.

3. INDEPENDENT CLAIM 1

D1 (Figs. 1, 2) is considered to represent the closest prior art and to disclose all features of claim 1 except:

i) means for focusing the emission beam onto the detector.
In D1 the vials are sequentially focused via optical fibres and lenses onto a grating and then by reflection to a detector.

Re i): D2 (Fig. 15) which lies in the same field as D1 discloses focusing of a sample array onto a CCD camera 16a' to permit parallel processing of the fluorescence signals - the output from individual reaction chambers is isolated by identifying respective pixel regions in the CCD array. The skilled person would modify the device of D1 from sequential detection of the vials to simultaneous detection in order to increase throughput and this would require merely

replacement of the optics used above the array of vials.

Independent claim 1 therefore does not meet the requirement of inventive step (Art. 33. 3 PCT).

4. INDEPENDENT CLAIM 27

In view of the disclosure of D1 referred to in the discussion of claim 1 above, claim 27 does not meet the requirement of novelty (Art. 33.2 PCT).

5. INDEPENDENT CLAIM 29

D2 EP0640828 (Fig. 15) is considered to represent the closest prior art. This document discloses the use, in a DNA PCR apparatus, of a plurality of filters - one for the excitation light mounted at an aperture in the housing and others arranged for interchangeable interception of the emission beams such as on a filter wheel coupled to a camera. In this way, different emission wavelengths and thus nucleic acid sequences in a given sample can be monitored and the presence of the target sequence can be determined.

D2 therefore discloses all features of claim 29 except the integration of excitation filter, emission filter and beam splitter into a single module and the provision of a support frame into which this integrated module is inserted.

D3 US5792610 (Fig. 1) which also lies in the field of apparatus for use in monitoring polymerase chain reactions states that :"two INTERCHANGEABLE filters were chosen one for use with Cy5, Cy5.5 (Oriel #58893; 740 nm cutoff) and one for use with Cy7 (Oriel 58895; 790 nm cutoff)." and that, "switching the filter sets in excitation and emission path as well as the dichroic mirror was done manually, but a computer-controlled electro-mechanical solution will allow automation of the procedure". The replacement of the filter wheel in D2 by an automatically interchangeable module comprising dichroic mirror, excitation and emission filters would therefore be an obvious modification to the skilled person.

Claim 29 therefore does not meet the requirement of inventive step (Art. 33.3 PCT).

6. DEPENDENT CLAIMS 2-26; 28; 30

The additional features of the dependent claims listed below are evident from the prior art as indicated:

Claims 2-3, 5-6, 11, 28, 30. D1, Fig. 2.

Claim 4. D1 (Fig. 2 - vial lenses); D2 (Figs. 15, 6 - focusing of individual light beams onto detector array; parallel processing)

Claim 7. The choice of Fresnel lenses which offer short focal lengths would be obvious in view of the requirement of compactness of the instrument.

Claims 8;9, 10,14-15,19,22 , 25. D2 (Fig. 15 - focusing of individual light from beams from the vials onto a detector array); D1 discusses the use of internal fluorescent dyes and D2 the use of a control tube 26C with a fluorescence dye in the array of samples for compensating drift measurement. The use of a plurality of references from which the maximum intensity is selected, subject to avoidance of detector saturation, is the obvious way to ensure optimum accuracy of normalisation.

Claim 12. See discussion of claim 29.

Claim 13. D4 (Fig. 11) discloses an elliptical reflector with a Xenon source; alternative use of a Halogen in place of Xenon would be obvious as would the desirability of reflecting visible light whilst transmitting visible since only the former is useful as excitation light.

Claims 20, 21. D2 describes the use of a shutter to the light source to intermittently expose the reaction mixtures to the excitation light since otherwise photo-deactivation ("bleaching") of the sample can occur. Camera exposure

control of the CCD array which must be thermoelectrically cooled is also disclosed. The purpose of the exposure time control is to avoid saturation of the detector CCD array i.e. maintaining the energy received by the detector below a certain maximum limit.

Claims 23, 24, 26. The vial 26c in D2 contains reference fluorescent dye but no DNA for purpose of normalisation; D2 also discloses the technique in which, " the shutter is timed to expose the mixtures to the excitation light during the annealing/extension phase where maximum fluorescence generally can be exhibited" whereas the reference vial 26c is measured before the reaction. In D1 where an internal reference is used the target DNA is first denatured by heating, then cooled to a temperature that allows annealing and extension and repeatedly cycled. It would therefore be obvious to the skilled person to measure the sample and reference fluorescences at points in the cycle where they respectively give their strongest signals

The subject-matter of claims 2-15, 19-26; 28; 30 therefore also does not meet the requirement of inventive step (Art. 33.3).

The subject-matter of claims 16-18 relating to the use of a plastic fluorescent strip as a reference and means for heating such are neither disclosed nor suggested by the cited documents of prior art so that these claims appear to meet the requirement of inventive step (Arts. 33.3 PCT).

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
AKER, David
PERMAN & GREEN, LLP
425 Post Road
Fairfield
Connecticut 06430-6232
ETATS-UNIS D'AMERIQUE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	01.03.2000
Applicant's or agent's file reference: 715-009111WO	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/11088	17/05/1999	16/05/1998

International Patent Classification (IPC) or both national classification and IPC
G01N21/25

Applicant
THE PERKIN-ELMER CORPORATION et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 16/09/2000.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Mason, W

Formalities officer (incl. extension of time limits)
Conner, M
Telephone No. +49 89 2399 2241

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-18 as originally filed

Claims, No.:

1-30 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	27
Inventive step (IS)	Claims	1-15,19-26,28-30
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

WITH RESPECT TO POINT V

1. The following documents are referred to in this opinion

D1=US5736333; D2=EP0640828; D3=US5792610; D4=WO9746707

The present application relates to optical apparatus for monitoring polymerase chain reactions for replication of DNA held in vials of a thermal cycler block. The apparatus comprises arrangement of light sources, filters, detectors, beam splitters, and lenses in particular arrangements.

2. PRELIMINARY

In as much as any of the claims are directed to apparatus which does not contain features relating to signals representative of the DNA sample, such claimed apparatus is not limited by the form of the sample itself (DNA replication fluids) and prior art disclosing the same apparatus features for samples of a different type must still be considered relevant.

3. INDEPENDENT CLAIM 1

D1 (Figs. 1, 2) is considered to represent the closest prior art and to disclose all features of claim 1 except:

i) means for focusing the emission beam onto the detector.
In D1 the vials are sequentially focused via optical fibres and lenses onto a grating and then by reflection to a detector.

Re i): D2 (Fig. 15) which lies in the same field as D1 discloses focusing of a sample array onto a CCD camera 16a' to permit parallel processing of the fluorescence signals - the output from individual reaction chambers is isolated by identifying respective pixel regions in the CCD array. The skilled person would modify the device of D1 from sequential detection of the vials to simultaneous detection in order to increase throughput and this would require merely

replacement of the optics used above the array of vials.

Independent claim 1 therefore does not meet the requirement of inventive step (Art. 33. 3 PCT).

4. INDEPENDENT CLAIM 27

In view of the disclosure of D1 referred to in the discussion of claim 1 above, claim 27 does not meet the requirement of novelty (Art. 33.2 PCT).

5. INDEPENDENT CLAIM 29

D2 EP0640828 (Fig. 15) is considered to represent the closest prior art. This document discloses the use, in a DNA PCR apparatus, of a plurality of filters - one for the excitation light mounted at an aperture in the housing and others arranged for interchangeable interception of the emission beams such as on a filter wheel coupled to a camera. In this way, different emission wavelengths and thus nucleic acid sequences in a given sample can be monitored and the presence of the target sequence can be determined.

D2 therefore discloses all features of claim 29 except the integration of excitation filter, emission filter and beam splitter into a single module and the provision of a support frame into which this integrated module is inserted.

D3 US5792610 (Fig. 1) which also lies in the field of apparatus for use in monitoring polymerase chain reactions states that :"two INTERCHANGEABLE filters were chosen one for use with Cy5, Cy5.5 (Oriel #58893; 740 nm cutoff) and one for use with Cy7 (Oriel 58895; 790 nm cutoff)." and that, "switching the filter sets in excitation and emission path as well as the dichroic mirror was done manually, but a computer-controlled electro-mechanical solution will allow automation of the procedure". The replacement of the filter wheel in D2 by an automatically interchangeable module comprising dichroic mirror, excitation and emission filters would therefore be an obvious modification to the skilled person.

Claim 29 therefore does not meet the requirement of inventive step (Art. 33.3 PCT).

6. DEPENDENT CLAIMS 2-26; 28; 30

The additional features of the dependent claims listed below are evident from the prior art as indicated:

Claims 2-3, 5-6, 11, 28, 30. D1, Fig. 2.

Claim 4. D1 (Fig. 2 - vial lenses); D2 (Figs. 15, 6 - focusing of individual light beams onto detector array; parallel processing)

Claim 7. The choice of Fresnel lenses which offer short focal lengths would be obvious in view of the requirement of compactness of the instrument.

Claims 8;9, 10,14-15,19,22 , 25. D2 (Fig. 15 - focusing of individual light from beams from the vials onto a detector array); D1 discusses the use of internal fluorescent dyes and D2 the use of a control tube 26C with a fluorescence dye in the array of samples for compensating drift measurement. The use of a plurality of references from which the maximum intensity is selected, subject to avoidance of detector saturation, is the obvious way to ensure optimum accuracy of normalisation.

Claim 12. See discussion of claim 29.

Claim 13. D4 (Fig. 11) discloses an elliptical reflector with a Xenon source; alternative use of a Halogen in place of Xenon would be obvious as would the desirability of reflecting visible light whilst transmitting visible since only the former is useful as excitation light.

Claims 20, 21. D2 describes the use of a shutter to the light source to intermittently expose the reaction mixtures to the excitation light since otherwise

photo-deactivation ("bleaching") of the sample can occur. Camera exposure control of the CCD array which must be thermoelectrically cooled is also disclosed. The purpose of the exposure time control is to avoid saturation of the detector CCD array i.e. maintaining the energy received by the detector below a certain maximum limit.

Claims 23, 24, 26. The vial 26c in D2 contains reference fluorescent dye but no DNA for purpose of normalisation; D2 also discloses the technique in which, " the shutter is timed to expose the mixtures to the excitation light during the annealing/extension phase where maximum fluorescence generally can be exhibited" whereas the reference vial 26c is measured before the reaction. In D1 where an internal reference is used the target DNA is first denatured by heating, then cooled to a temperature that allows annealing and extension and repeatedly cycled. It would therefore be obvious to the skilled person to measure the sample and reference fluorescences at points in the cycle where they respectively give their strongest signals

The subject-matter of claims 2-15, 19-26; 28; 30 therefore also does not meet the requirement of inventive step (Art. 33.3).

The subject-matter of claims 16-18 relating to the use of a plastic fluorescent strip as a reference and means for heating such are neither disclosed nor suggested by the cited documents of prior art so that these claims appear to meet the requirement of inventive step (Arts. 33.3 PCT).

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D-80298 München
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FAX +49 89 2399-4465

Correspondence with the EPO on PCT Chapter II demands

In order to ensure that your PCT Chapter II demand is dealt with as promptly as possible you are requested to use the enclosed self-adhesive labels with any correspondence relating to the demand sent to the Munich Office.

One of these labels should be affixed to a prominent place in the upper part of the letter or form etc. which you are filing.

6

PATENT COOPERATION TREATY

REC'D 18 AUG 2000

WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
715-009111WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/11088	17/05/1999	16/05/1998
International Patent Classification (IPC) or national classification and IPC		
G01N21/25		
Applicant		
THE PERKIN-ELMER CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/12/1999	16.08.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mason, W Telephone No. +49 89 2399 2623

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/11088

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-18 as originally filed

Claims, No.:

1-30 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-26,28-30
	No:	Claims	27
Inventive step (IS)	Yes:	Claims	16-18
	No:	Claims	1-15,19-26,28-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations

see separate sheet

WITH RESPECT TO POINT V

1. The following documents are referred to in this report:

   D1=US5736333; D2=EP0640828; D3=US5792610; D4=WO9746707

   The present application relates to optical apparatus for monitoring polymerase chain reactions for replication of DNA held in vials of a thermal cycler block. The apparatus comprises arrangement of light sources, filters, detectors, beam splitters, and lenses in particular arrangements.

2. PRELIMINARY

   In as much as any of the claims are directed to apparatus which does not contain features relating to signals representative of the DNA sample, such claimed apparatus is not limited by the form of the sample itself (DNA replication fluids) and prior art disclosing the same apparatus features for samples of a different type must still be considered relevant.

3. INDEPENDENT CLAIM 1

   D1 (Figs. 1, 2) is considered to represent the closest prior art and to disclose all features of claim 1 except:

   i) means for focusing the emission beam onto the detector.
   In D1 the vials are sequentially focused via optical fibres and lenses onto a grating and then by reflection to a detector.

   Re i): D2 (Fig. 15) which lies in the same field as D1 discloses focusing of a sample array onto a CCD camera 16a' to permit parallel processing of the fluorescence signals - the output from individual reaction chambers is isolated by identifying respective pixel regions in the CCD array. The skilled person would modify the device of D1 from sequential detection of the vials to simultaneous detection in order to increase throughput and this would require merely

replacement of the optics used above the array of vials.

Independent claim 1 therefore does not meet the requirement of inventive step (Art. 33. 3 PCT).

4. INDEPENDENT CLAIM 27

In view of the disclosure of D1 referred to in the discussion of claim 1 above, claim 27 does not meet the requirement of novelty (Art. 33.2 PCT).

5. INDEPENDENT CLAIM 29

D2 EP0640828 (Fig. 15) is considered to represent the closest prior art. This document discloses the use, in a DNA PCR apparatus, of a plurality of filters - one for the excitation light mounted at an aperture in the housing and others arranged for interchangeable interception of the emission beams such as on a filter wheel coupled to a camera. In this way, different emission wavelengths and thus nucleic acid sequences in a given sample can be monitored and the presence of the target sequence can be determined.

D2 therefore discloses all features of claim 29 except the integration of excitation filter, emission filter and beam splitter into a single module and the provision of a support frame into which this integrated module is inserted.

D3 US5792610 (Fig. 1) which also lies in the field of apparatus for use in monitoring polymerase chain reactions states that :"two INTERCHANGEABLE filters were chosen one for use with Cy5, Cy5.5 (Oriel #58893; 740 nm cutoff) and one for use with Cy7 (Oriel 58895; 790 nm cutoff)." and that, "switching the filter sets in excitation and emission path as well as the dichroic mirror was done manually, but a computer-controlled electro-mechanical solution will allow automation of the procedure". The replacement of the filter wheel in D2 by an automatically interchangeable module comprising dichroic mirror, excitation and emission filters would therefore be an obvious modification to the skilled person.

Claim 29 therefore does not meet the requirement of inventive step (Art. 33.3 PCT).

6. DEPENDENT CLAIMS 2-26; 28; 30

The additional features of the dependent claims listed below are evident from the prior art as indicated:

Claims 2-3, 5-6, 11, 28, 30. D1, Fig. 2.

Claim 4. D1 (Fig. 2 - vial lenses); D2 (Figs. 15, 6 - focusing of individual light beams onto detector array; parallel processing)

Claim 7. The choice of Fresnel lenses which offer short focal lengths would be obvious in view of the requirement of compactness of the instrument.

Claims 8;9, 10,14-15,19,22 , 25. D2 (Fig. 15 - focusing of individual light from beams from the vials onto a detector array); D1 discusses the use of internal fluorescent dyes and D2 the use of a control tube 26C with a fluorescence dye in the array of samples for compensating drift measurement. The use of a plurality of references from which the maximum intensity is selected, subject to avoidance of detector saturation, is the obvious way to ensure optimum accuracy of normalisation.

Claim 12. See discussion of claim 29.

Claim 13. D4 (Fig. 11) discloses an elliptical reflector with a Xenon source; alternative use of a Halogen in place of Xenon would be obvious as would the desirability of reflecting visible light whilst transmitting visible since only the former is useful as excitation light.

Claims 20, 21. D2 describes the use of a shutter to the light source to intermittently expose the reaction mixtures to the excitation light since otherwise photo-deactivation ("bleaching") of the sample can occur. Camera exposure

control of the CCD array which must be thermoelectrically cooled is also disclosed. The purpose of the exposure time control is to avoid saturation of the detector CCD array i.e. maintaining the energy received by the detector below a certain maximum limit.

Claims 23, 24, 26. The vial 26c in D2 contains reference fluorescent dye but no DNA for purpose of normalisation; D2 also discloses the technique in which, " the shutter is timed to expose the mixtures to the excitation light during the annealing/extension phase where maximum fluorescence generally can be exhibited" whereas the reference vial 26c is measured before the reaction. In D1 where an internal reference is used the target DNA is first denatured by heating, then cooled to a temperature that allows annealing and extension and repeatedly cycled. It would therefore be obvious to the skilled person to measure the sample and reference fluorescences at points in the cycle where they respectively give their strongest signals

The subject-matter of claims 2-15, 19-26; 28; 30 therefore also does not meet the requirement of inventive step (Art. 33.3).

The subject-matter of claims 16-18 relating to the use of a plastic fluorescent strip as a reference and means for heating such are neither disclosed nor suggested by the cited documents of prior art so that these claims appear to meet the requirement of inventive step (Arts. 33.3 PCT).

T 5

VERTRAG ÜBER[redacted]: INTERNATIONALE ZUSA[redacted]ENARBEIT AUF DEM GEBIET DES PATENTWESENS

PCT

INTERNATIONALER VORLÄUFIGER PRÜFUNGSBERICHT

(Artikel 36 und Regel 70 PCT)

Aktenzeichen des Anmelders oder Anwalts	**WEITERES VORGEHEN**	siehe Mitteilung über die Übersendung des internationalen vorläufigen Prüfungsberichts (Formblatt PCT/IPEA/416)
P 50055 V		
Internationales Aktenzeichen	Internationales Anmeldedatum *(Tag/Monat/Jahr)*	Prioritätsdatum *(Tag/Monat/Tag)*
PCT/EP00/03420	14/04/2000	23/04/1999
Internationale Patentklassifikation (IPK) oder nationale Klassifikation und IPK B01F9/00		
Anmelder VASCULAR BIOTECH GMBH et al.		

1. Dieser internationale vorläufige Prüfungsbericht wurde von der mit der internationalen vorläufigen Prüfung beauftragten Behörde erstellt und wird dem Anmelder gemäß Artikel 36 übermittelt.

2. Dieser BERICHT umfaßt insgesamt 4 Blätter einschließlich dieses Deckblatts.

   ☐ Außerdem liegen dem Bericht ANLAGEN bei; dabei handelt es sich um Blätter mit Beschreibungen, Ansprüchen und/oder Zeichnungen, die geändert wurden und diesem Bericht zugrunde liegen, und/oder Blätter mit vor dieser Behörde vorgenommenen Berichtigungen (siehe Regel 70.16 und Abschnitt 607 der Verwaltungsrichtlinien zum PCT).

   Diese Anlagen umfassen insgesamt Blätter.

3. Dieser Bericht enthält Angaben zu folgenden Punkten:

   - I ☒ Grundlage des Berichts
   - II ☐ Priorität
   - III ☐ Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit
   - IV ☐ Mangelnde Einheitlichkeit der Erfindung
   - V ☒ Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderischen Tätigkeit und der gewerblichen Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung
   - VI ☐ Bestimmte angeführte Unterlagen
   - VII ☐ Bestimmte Mängel der internationalen Anmeldung
   - VIII ☐ Bestimmte Bemerkungen zur internationalen Anmeldung

Datum der Einreichung des Antrags	Datum der Fertigstellung dieses Berichts
21/11/2000	02.02.2001
Name und Postanschrift der mit der internationalen vorläufigen Prüfung beauftragten Behörde: Europäisches Patentamt D-80298 München Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bevollmächtigter Bediensteter Tiercet, M Tel. Nr. +49 89 2399 8977

INTERNATIONALER VORLÄUFIGER PRÜFUNGSBERICHT

Internationales Aktenzeichen PCT/EP00/03420

I. Grundlage des Berichts

1. Dieser Bericht wurde erstellt auf der Grundlage (*Ersatzblätter, die dem Anmeldeamt auf eine Aufforderung nach Artikel 14 hin vorgelegt wurden, gelten im Rahmen dieses Berichts als "ursprünglich eingereicht" und sind ihm nicht beigefügt, weil sie keine Änderungen enthalten.*):
Beschreibung, Seiten:

1-21 ursprüngliche Fassung

Patentansprüche, Nr.:

1-24 ursprüngliche Fassung

Zeichnungen, Blätter:

1/3-3/3 ursprüngliche Fassung

2. Hinsichtlich der **Sprache:** Alle vorstehend genannten Bestandteile standen der Behörde in der Sprache, in der die internationale Anmeldung eingereicht worden ist, zur Verfügung oder wurden in dieser eingereicht, sofern unter diesem Punkt nichts anderes angegeben ist.

   Die Bestandteile standen der Behörde in der Sprache: zur Verfügung bzw. wurden in dieser Sprache eingereicht; dabei handelt es sich um

   ☐ die Sprache der Übersetzung, die für die Zwecke der internationalen Recherche eingereicht worden ist (nach Regel 23.1(b)).

   ☐ die Veröffentlichungssprache der internationalen Anmeldung (nach Regel 48.3(b)).

   ☐ die Sprache der Übersetzung, die für die Zwecke der internationalen vorläufigen Prüfung eingereicht worden ist (nach Regel 55.2 und/oder 55.3).

3. Hinsichtlich der in der internationalen Anmeldung offenbarten **Nucleotid- und/oder Aminosäuresequenz** ist die internationale vorläufige Prüfung auf der Grundlage des Sequenzprotokolls durchgeführt worden, das:

   ☐ in der internationalen Anmeldung in schriftlicher Form enthalten ist.

   ☐ zusammen mit der internationalen Anmeldung in computerlesbarer Form eingereicht worden ist.

   ☐ bei der Behörde nachträglich in schriftlicher Form eingereicht worden ist.

   ☐ bei der Behörde nachträglich in computerlesbarer Form eingereicht worden ist.

   ☐ Die Erklärung, daß das nachträglich eingereichte schriftliche Sequenzprotokoll nicht über den Offenbarungsgehalt der internationalen Anmeldung im Anmeldezeitpunkt hinausgeht, wurde vorgelegt.

   ☐ Die Erklärung, daß die in computerlesbarer Form erfassten Informationen dem schriftlichen Sequenzprotokoll entsprechen, wurde vorgelegt.

4. Aufgrund der Änderungen sind folgende Unterlagen fortgefallen:

- ☐ Beschreibung, Seiten:
- ☐ Ansprüche, Nr.:
- ☐ Zeichnungen, Blatt:

5. ☐ Dieser Bericht ist ohne Berücksichtigung (von einigen) der Änderungen erstellt worden, da diese aus den angegebenen Gründen nach Auffassung der Behörde über den Offenbarungsgehalt in der ursprünglich eingereichten Fassung hinausgehen (Regel 70.2(c)).

   (Auf Ersatzblätter, die solche Änderungen enthalten, ist unter Punkt 1 hinzuweisen;sie sind diesem Bericht beizufügen).

6. Etwaige zusätzliche Bemerkungen:

V. Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderischen Tätigkeit und der gewerblichen Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung

1. Feststellung

Neuheit (N)	Ja:	Ansprüche	1-24
	Nein:	Ansprüche	
Erfinderische Tätigkeit (ET)	Ja:	Ansprüche	1-24
	Nein:	Ansprüche	
Gewerbliche Anwendbarkeit (GA)	Ja:	Ansprüche	1-24
	Nein:	Ansprüche	

2. Unterlagen und Erklärungen
   siehe Beiblatt

Gegenüber dem nächstliegenden Stand der Technik, dem Dokument WO-A-9301843, das ein System nach Anspruch 1 beschreibt, das aus einem mit Endkappen, Klemmdichtungen und Kanülen versehenen Rohr besteht, weist Anspruch 1 die neuen Merkmale auf, daß das Innerohr Löcher aufweist, durch die ein Kulturmedium einfüllbar ist, und daß die Gasversorgung des Rohrzwischenraums durch eine um das Innerohr geführte gasdurchlässige Leitung erfolgt. Die neuen Merkmale erlauben Sterilität ohne Inanspruchnahme von Reinst-Räumen bei Beschichtungen von Spenderblutgefässen. Keines der im Recherchenbericht zitierten Dokumente vermag die neuen Merkmale nahezulegen, so daß Anspruch 1 als nicht offensichtliche Alternative die Bedingungen von Artikel 33(2)und 33(3) PCT erfüllt. Das gleiche gilt für den rückbezogenen Verfahrensanspruch 24. Die gewerbliche Anwendbarkeit erscheint offensichtlich.

The demand must be filed directly with the competent International Preliminary Examining Authority. If two or more Authorities are competent, with the one chosen by the applicant. The name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

Copy

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 715-009111WO

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US99/11088	17 May 1999 (17.05.99)	16 May 1998 (16.05.98)

Title of invention: INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

The Perkin-Elmer Corporation
761 Main Avenue
Norwalk, Connecticut 06859-0199
United States of America

Telephone No.: 203-761-5123
Facsimile No.: 203-761-2888
Teleprinter No.:

State *(that is, country)* of nationality: US
State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GAMBINI, Michael R.
181 Josiesing Road
Monroe, Connecticut 06468
United States of America

State *(that is, country)* of nationality: US
State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ATWOOD, John G.
149 Limekiln Road
Redding, CT 06896
United States of America

State *(that is, country)* of nationality: US
State *(that is, country)* of residence: US

[X] Further applicants are indicated on a continuation sheet.

International application No.
PCT/US99/11088

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet is not to be included in the demand

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
YOUNG, Eugene F.
802 Balboa Lane
Foster City, California 94404
United States of America

State *(i.e. country)* of nationality:
US

State *(i.e. country)* of residence:
US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
LAKATOS, Edward J.
56 Ridgedale Road
Bethel, Connecticut 06801
United States of America

State *(i.e. country)* of nationality:
US

State *(i.e. country)* of residence:
US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
CERRONE, Anthony L.
51 Kneeland Road
New Haven, Connecticut
United States of America

State *(i.e. country)* of nationality:
US

State *(i.e. country)* of residence:
US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/US99/11088

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

AKER, David
Perman & Green, LLP
425 Post Road
Fairfield, Connecticut 06430-6232
United States of America

Telephone No.: 203-259-1800

Facsimile No.: 203-255-5170

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[X] the international application as originally filed

the description [X] as originally filed
[ ] as amended under Article 34

the claims [XX] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

Sheet No. 3

International application No.
PCT/US99/11088

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:		sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter Request for Change in Correspondence Address	:	1	sheets	☐	☐
6. other *(specify)* Copy of Power of Attorney	:	3	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

David Aker (Agent)

David Aker

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PCT

CHAPTER II

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No.	PCT/US99/11088
Applicant's or agent's file reference	PCT 1108-031/ld

For International Preliminary Examining Authority use only

Date stamp of the IPEA

Applicant
THE PERKIN-ELMER CORPORATION

Calculation of prescribed fees

1. Preliminary examination fee ..... EUR 1.533,00 P

2 Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)* ..... EUR 148,00 H

3. Total of prescribed fees
Add the amounts entered at P and H and enter total in the TOTAL box ..... EUR 1.681,00
TOTAL

Mode of Payment

☒	authorization to charge deposit account with the IPEA (see below)	☐	cash
☐	cheque	☐	revenue stamps
☐	postal money order	☐	coupons
☐	bank draft	☐	other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA /EP ☒ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

28 00 04 37	15.12.99	Dr. rer. nat. A. Pfau
Deposit Account Number	Date *(day/month/year)*	Signature

See Notes to the fee calculation sheet

The demand must be filed directly with the competent International Preliminary Examining Authority. If two or more Authorities are competent, with the one chosen by the applicant. The name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ EP

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
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For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 715-009111WO

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/US99/11088	17 May 1999 (17.05.99)	16 May 1998 (16.05.98)

Title of invention: INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

The Perkin-Elmer Corporation
761 Main Avenue
Norwalk, Connecticut 06859-0199
United States of America

Telephone No.: 203-761-5123
Facsimile No.: 203-761-2888
Teleprinter No.:

State *(that is, country)* of nationality: US
State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:
State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:
State *(that is, country)* of residence:

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/US99/11088

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

AKER, David
Perman & Green, LLP
425 Post Road
Fairfield, Connecticut 06430-6232
United States of America

Telephone No.: 203-259-1800

Facsimile No.: 203-255-5170

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[X] the international application as originally filed

the description [X] as originally filed
[ ] as amended under Article 34

the claims [XX] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

PCT

POWER OF ATTORNEY

(for an international application filed under the Patent Cooperation Treaty)

(PCT Rule 90.4)

The undersigned applicant(s) *(Names should be indicated as they appear in the request)*:

The Perkin-Elmer Corporation
761 Main Avenue
Norwalk, Connecticut 06859-0199
United States of America

hereby appoints (appoint) the following person as: [XX] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

AKER, David
GREEN, Clarence A.
HARRINGTON, Mark A.
PERMAN & GREEN, LLP
425 Post Road
Fairfield, Connecticut 06430
United States of America

to represent the undersigned before

[XX] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

Applicant's or agent's file reference: 715-009111-WO

International application number (if already available): PCT/US99/11088

filed with the following Office US as receiving Office
and to make or receive payments on behalf of the undersigned.

Signature of the applicant(s) *(where there are several applicants, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading the request or this power)*:

_____(signature)

_____(name typed)

_____(title)

Date: _____

Form PCT/Model of power of attorney (for a given international application) (July 1992)

(Form PCT/Model of Power of Attorney [13-3.2])

Sheet No. 3

International application No.
PCT/US99/11088

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:				For International Preliminary Examining Authority use only	
				received	not received
1. translation of international application	:		sheets	☐	☐
2. amendments under Article 34	:		sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:		sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:		sheets	☐	☐
5. letter Request for Change in Correspondence Address	:	1	sheets	☐	☐
6. other *(specify)* Copy of Power of Attorney	:	3	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

David Aker (Agent)

David Aker

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

DAVID AKER
THE PERKIN-ELMER CORPORATION
761 MAIN AVENUE
NORWALK CT 06859-0199

PCT

NOTIFICATION CONCERNING PAYMENT OF PRESCRIBED FEES

(PCT Rules 14, 15 and 16 and Administrative Instructions, Sections 304(a) and (b) and 323(b))

Date of mailing (*day/month/year*)	21 JUN 1999
Applicant's or agent's file reference: BT-4584/PCT	**PAYMENT DUE** See item 3 for time limits

International application No.	International filing date/Date of receipt (*day/month/year*)	Priority date (*day/month/year*)
PCT/US99/11088	17 MAY 99	16 MAY 98

Applicant: THE PERKIN-ELMER CORPORATION

1. The applicant is hereby notified that this receiving Office has received:

☑ **the payment** of all the prescribed fees, and ☐ **an overpayment**, which will be refunded in due course.

☐ **no or insufficient payment** of the prescribed fees and the applicant is hereby **invited to pay the balance due**, as summarized under item 2, within the time limit(s) indicated under item 3.

2. **Fees and payment calculation:**

_____ (Total fees payable) - _____ (Amount paid) = _____ (Balance)

☐ The details of the calculation are given in the Annex.

3. **Time limit(s) for payment and amount(s) payable (Rules 14.1, 15.4 and 16.1(f)):**

☐ within ONE MONTH from the date of receipt of the international application **(for the transmittal fee** (if any), **the search fee, the basic fee** and **the designation fee)**. The amount payable for each fee is the amount applicable on the date of receipt of the international application.

☐ within ONE YEAR from the priority date (**only for the designation fee** and only if this time limit expires later than the above time limit).
---If the designation fee is paid within one month from the date of receipt of the international application, the amount payable is the amount applicable on that date of receipt.
---If the designation fee is paid within one year from the priority date but later than one month from the date of receipt of the international application, the amount payable is the amount applicable on the date of payment. The receiving Office should be consulted for the applicable amount.

☐ within 16 MONTHS from the priority date (only for the fee for priority document). The applicant's attention is drawn to the fact that the request made by the applicant under Rule 17.1(b) will be considered not to have been made unless the fee is paid within that time limit.

4. Additional observations *(if necessary)*:

☐ The search copy will not be transmitted to the International Searching Authority until the search fee is paid (therefore the start of the international search will be delayed)(Rule 23.1(a) and (b)).

Name and mailing address of the receiving Office Assistant Commissioner for Patents Box PCT Washington, D.C. 20231 Attn: RO/US Facsimile No.	Authorized officer Georgette Hill PCT INTERNATIONAL SERVICES DIVISION Telephone No.

RECEIVED JUN 24 1999

Form PCT/RO/102 (January 1999)

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

PCT

To:

DAVID AKER
THE PERKIN-ELMER CORPORATION
761 MAIN AVENUE
NORWALK CT 06859-0199

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing (*day/month/year*): 21 JUN 1999

Applicant's or agent's file reference: BT-4584/PCT

IMPORTANT NOTIFICATION

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US99/11088	17 MAY 99	16 MAY 98

Applicant: THE PERKIN-ELMER CORPORATION

Title of the invention: INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

☑ was transmitted to the International Bureau on 21 JUN 1999

☐ has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

☐ because the necessary national security clearance has not yet been obtained.

☐ because (*reason to be specified*):

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

3. FOREIGN TRANSMITTAL LICENSE INFORMATION — Completed by: [illegible]

☐ Additional license for foreign transmittal not required. This subject matter is covered by a license already granted on the equivalent U.S. national application. Refer to that license for information concerning its scope.

☐ License for foreign transmittal not required. 37 CFR 5.11(e)(1) or 37 CFR 5.11(e)(2). However, a license may be required for additional subject matter. See 37 CFR 5.15(b).

☑ Foreign transmittal license granted. 35 U.S.C. 184; 37 CFR 5.11 on 5-28-99 (date):

☑ 37 CFR 5.15(a) ☐ 37 CFR 5.15(b)

Name and mailing address of the receiving Office
Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231 Attn: RO/US
Facsimile No.

Authorized officer: Georgette Hill
PCT INTERNATIONAL SERVICES DIVISION
Telephone No. 703-305-3740

Form PCT/RO/105 (July 1992)

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

DAVID AKER
THE PERKIN-ELMER CORPORATION
761 MAIN AVENUE
NORWALK CT 06859-0199

PCT

INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

(PCT Articles 3(4)(i) and 14(1) and Rule 26)

Applicant's or agent's file reference BT-4584/PCT	Date of mailing (*day/month/year*) 21 JUN 1999
International application No. PCT/US99/11088	**REPLY DUE** within **ONE MONTH** from the above date of mailing
Applicant THE PERKIN-ELMER CORPORATION	International filing date (*day/month/year*) 17 MAY 99

1. ☒ The applicant is hereby invited, within the time limit indicated above, to correct, **in the international application as filed,** the defects specified on the attached
   - ☒ Annex A
   - ☐ Annex B1 *(text matter of the international application as filed)*
   - ☒ Annex C1 *(drawings of the international application as filed)*

2. ☐ The applicant is hereby invited, within the time limit indicated above, to correct, **in the translation of the international application** furnished under Rule 12.3, the defects specified on the attached
   - ☐ Annex A
   - ☐ Annex B2 *(text matter of the translation of the international application)*
   - ☐ Annex C2 *(drawings of the translation of the international application)*

Additional observations (if necessary):

HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (Rule 26.4).

ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachments has been sent to the International Bureau ☒ and the International Searching Authority.

Name and mailing address of the receiving Office Assistant Commissioner for Patents Box PCT Washington, D.C. 20231 Attn: RO/US Facsimile No.	Authorized officer Georgette Hill PCT INTERNATIONAL SERVICES DIVISION Telephone No.

Form PCT/RO/106 (July 1998)

ANNEX A TO FORM PCT/RO/106

International application No.
PCT/US97/11088

The receiving Office has found the following defects in the international application:

1. As to signature* of the international application (Rules 4.15 and 90.4), the request:
   a. ☐ is not signed.
   b. ☒ is not signed by all the applicants.
   c. ☐ is not accompanied by the statement referred to in the check list in Box No. VIII of the request explaining the lack of the signature of an applicant for the designation of the United States of America.
   d. ☐ is signed by what appears to be an agent/common representative but
      ☐ the international application is not accompanied by a power of attorney appointing him.
      ☐ the power of attorney accompanying the international application was not signed by all the applicants.
   e. ☐ other *(specify)*:

* All applicants must sign, including inventors if they are also applicants (e.g. where the United States of America is designated).

2. As to indications concerning the applicant, the request (Rules 4.4 and 4.5):
   a. ☐ does not properly indicate the applicant's name *(specify)*:
   b. ☐ does not indicate the applicant's address.
   c. ☐ does not properly indicate the applicant's address *(specify)*:
   d. ☐ does not indicate the applicant's nationality.
   e. ☐ does not indicate the applicant's residence.
   f. ☐ other *(specify)*:

3. As to the language of some parts of the international application (Rule 12.1):
   a. ☐ the request is not in (one of) the admitted language(s) which is (are): _____
   b. ☐ the text matter of the drawings is not in (one of) the admitted language(s) which is (are): _____
   c. ☐ the abstract is not in (one of) the admitted language(s) which is (are): _____

4. The title of the invention:
   a. ☐ is not indicated in Box No. 1 of the request (Rule 4.1(a)).
   b. ☐ is not indicated at the top of the first sheet of the description (Rule 5.1(a)).
   c. ☐ as appearing in Box No. 1 of the request is not identical with the title heading the description (Rule 5.1(a)).

5. As to the abstract (Rule 8):
   ☐ the international application does not contain an abstract.

ANNEX TO FORM PCT/RO/106

99/11058

The receiving Office has found that, with regard to the presentation of the **drawings of the international application as filed**, the physical requirements are not complied with to the extent that compliance therewith is necessary for:

1. ☐ reasonally uniform international publication (Rules 11 and 26.3(a)(i)) *(defects to be specified)*

Sheets containing drawings:

a. ☐ the sheets do not admit of direct reproduction.
b. ☐ the sheets are not free from creases, cracks, folds.
c. ☐ one side of the sheets is not left unused.
d. ☐ the paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable.
e. ☐ the drawings do not commence on a new sheet.
f. ☐ the sheets are not connected as prescribed (Rule 11.4(b)).
g. ☐ the sheets are not A4 size (29.7cm x 21cm).
h. ☐ the minimum margins on the sheets are not as prescribed (top: 2.5cm; left side: 2.5cm; right side: 1.5cm; bottom: 1cm).
i. ☐ the file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5cm of the top of the sheets.
j. ☐ the file reference number exceeds the maximum of 12 characters.
k. ☐ the sheets are not free from frames around usable or used surfacts.
l. ☒ the sheets are not numbered in consecutive Arabic numerals (e.g. 1/3, 2/3, 3/3). ALL
m. ☐ the sheet numbers are not centered at the top or bottom of the sheets.
n. ☐ the sheet numbers are in the margin (see h. above for the size of the margins).
o. ☐ the sheets contain alterations/overwritings/interlineations/too many erasures.
p. ☒ the sheets contain photocopy marks. FIG. 1-9

Drawings (Rule 11.13):

a. ☐ do not admit of direct reproduction.
b. ☐ contain unnecessary text matter.
c. ☐ contain words so placed as to prevent translation without interference with lines thereof.
d. ☒ are not executed in durable black color; the lines are not uniformly thick and well-defined. FIG. 1-6,9,8
e. ☐ contain cross-sections not properly hatched.
f. ☐ would not be properly distinguishable in reduced reproduction.
g. ☐ contain scales not represented graphically.
h. ☒ contain numbers, letters and reference lines lacking simplicity and clarity. FIG. 1,5,9,8
i. ☐ contain lines drafted without the aid of drafting instruments.
j. ☐ contain disproportionate elements of a figure not necessary for clarity.
k. ☐ contain numbers and letters of height less than 0.32 cm.
l. ☐ contain letters not conforming to the Latin, and where customary, Greek alphabets.
m. ☐ contain figures on two or more sheets which form a single complete figure but which are not able to be assembled without concealing parts thereof.
n. ☐ contain figures which are not properly arranged and clearly separated.
o. ☐ contain different figures not numbered in consecutive Arabic numerals.
p. ☐ contain different figures not numbered independent of the numbering of the sheets.
q. ☐ are not restricted to reference signs mentioned in the description.
r. ☐ do not contain reference signs that are mentioned in the description.
s. ☐ contain the same feature denoted by different reference signs.
t. ☐ are not arranged in an upright position, clearly separated from one another.
u. ☐ are not presented sideways with the top of the figures at the left side of the sheets.

2. ☐ satisfactory reproduction (Rules 11 and 26.3(b)(i)).

Further observations (if neccesary):

NEW DRWGS REQ'D

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
DAVID AKER
THE PERKIN-ELMER CORPORATION
761 MAIN AVENUE
NORWALK, CT 06859 0199

RECEIVED
ABD - LEGAL DEPT.
AUG 16 1999
DOCKETED: YES (NO)

PCT

COMMUNICATION REGARDING EXTENSION OF TIME LIMIT

(PCT Rule 26.2)

Applicant's or agent's file reference: BT-4584/PCT 4529 WO pds	Date of mailing (day/month/year): 09 AUG 1999
International application No.: PCT/US99/11088	IMPORTANT COMMUNICATION
Applicant: THE PERKIN-ELMER CORPORATION	International filing date (day/month/year): 17 MAY 99

1. In response to the applicant's request of 21 Jul 1999, the time limit for replying to:

   ☒ the Invitation to Correct Defects (PCT/RO/106)

   ☐ (other) _____

   has been extended as follows:

   ☒ extension of 1 months/days from 21 Jul 1999

   ☒ extension until 21 Aug 1999

2. ☐ No extension of the time limit is granted and the time limit remains as previously set.

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Dean Layman Telephone No. 703/ 305-3165

Form PCT/RO/138 (July 1992)

PATENT COOPERATION TREATY

RECEIVED
JAN 2 1 2000
PERMAN AND GREEN LLP

PCT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
AKER, David
PERMAN & GREEN, LLP
425 Post Road
Fairfield
Connecticut 06430-6232
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*: 18. 01. 00

Applicant's or agent's file reference: 715-009111WO

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 99/ 11088	17/05/1999	16/05/1998

Applicant: THE PERKIN-EIMER CORPORATION et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

   15/12/1999

2. This date of receipt is:

   - [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
   - [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
   - [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

   [ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/

European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 epmu d
Fax: (+49-89) 2399-4465

Authorized officer

COMTE C S J

Tel. (+49-89) 2399-8598

EUROPÄISCHES PATENTAMT · EUROPEAN PATENT OFFICE · OFFICE EUROPÉEN DES BREVETS

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)*

Box No. I TITLE OF INVENTION

INSTRUMENT FOR MONITORING POLYMERASE CHAIN REACTION OF DNA

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

THE PERKIN-ELMER CORPORATION
761 Main Avenue
Norwalk, Connecticut 06859-0199
United States of America

☐ This person is also inventor.

Telephone No.
203-761-5123

Facsimile No.
203-761-2888

Teleprinter No.

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

GAMBINI, Michael R.
181 Josiesing Road
Monroe, Connecticut 06468
United States of America

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

AKER, David
The Perkin-Elmer Corporation
761 Main Avenue
Norwalk, Connecticut
United States of America

Telephone No.
203-761-5123

Facsimile No.
203-761-2888

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

ATWOOD, John G.
149 Limekiln Road
Redding, CT 06896
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

YOUNG, Eugene F.
802 Balboa Lane
Foster City, California 94404
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

LAKATOS, Edward J.
56 Ridgedale Road
Bethel, Connecticut 06801
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

CERRONE, Anthony L.
51 Kneeland Road
New Haven, Connecticut
United States of America

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☐ **AP** **ARIPO Patent:** GH Ghana, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☐ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☐ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☐ **AL** Albania .....
- ☐ **AM** Armenia .....
- ☐ **AT** Austria .....
- ☒ **AU** Australia .....
- ☐ **AZ** Azerbaijan .....
- ☐ **BA** Bosnia and Herzegovina .....
- ☐ **BB** Barbados .....
- ☐ **BG** Bulgaria .....
- ☐ **BR** Brazil .....
- ☐ **BY** Belarus .....
- ☒ **CA** Canada .....
- ☐ **CH and LI** Switzerland and Liechtenstein .....
- ☒ **CN** China .....
- ☐ **CU** Cuba .....
- ☐ **CZ** Czech Republic .....
- ☐ **DE** Germany .....
- ☐ **DK** Denmark .....
- ☐ **EE** Estonia .....
- ☐ **ES** Spain .....
- ☐ **FI** Finland .....
- ☐ **GB** United Kingdom .....
- ☐ **GE** Georgia .....
- ☐ **GH** Ghana .....
- ☐ **HU** Hungary .....
- ☐ **IL** Israel .....
- ☐ **IS** Iceland .....
- ☒ **JP** Japan .....
- ☐ **KE** Kenya .....
- ☐ **KG** Kyrgyzstan .....
- ☐ **KP** Democratic People's Republic of Korea .....
- ☐ **KR** Republic of Korea .....
- ☐ **KZ** Kazakstan .....
- ☐ **LC** Saint Lucia .....
- ☐ **LK** Sri Lanka .....
- ☐ **LR** Liberia .....
- ☐ **LS** Lesotho .....
- ☐ **LT** Lithuania .....
- ☐ **LU** Luxembourg .....
- ☐ **LV** Latvia .....
- ☐ **MD** Republic of Moldova .....
- ☐ **MG** Madagascar .....
- ☐ **MK** The former Yugoslav Republic of Macedonia .....
- ☐ **MN** Mongolia .....
- ☐ **MW** Malawi .....
- ☐ **MX** Mexico .....
- ☐ **NO** Norway .....
- ☐ **NZ** New Zealand .....
- ☐ **PL** Poland .....
- ☐ **PT** Portugal .....
- ☐ **RO** Romania .....
- ☐ **RU** Russian Federation .....
- ☐ **SD** Sudan .....
- ☐ **SE** Sweden .....
- ☐ **SG** Singapore .....
- ☐ **SI** Slovenia .....
- ☐ **SK** Slovakia .....
- ☐ **SL** Sierra Leone .....
- ☐ **TJ** Tajikistan .....
- ☐ **TM** Turkmenistan .....
- ☐ **TR** Turkey .....
- ☐ **TT** Trinidad and Tobago .....
- ☐ **UA** Ukraine .....
- ☐ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☐ **UZ** Uzbekistan .....
- ☐ **VN** Viet Nam .....
- ☐ **YU** Yugoslavia .....
- ☐ **ZW** Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....
- ☐ .....
- ☐ .....
- ☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) US	16 May 1998	60/085,765	
item (2) US	14 July 1998	60/092,784	
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1) and (2)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / EP

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	4	sheets
2. description	18	sheets
3. claims	13	sheets
4. abstract	1	sheets
5. drawings	7	sheets
Total	43	sheets

This international application is accompanied by the item(s) marked below:

1. ☒ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. 1 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

David Aker
Agent

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

TENT COOPERATION TREAT

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF RECEIPT OF SEARCH COPY

(PCT Rule 25.1)

To:
The PERKIN-ELMER CORPORATION
Attn. AKER, David
761 Main Avenue
Norwalk, CT 06859-0181
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)* 16/07/1999

Applicant's or agent's file reference
BT-4584/PCT 4529 WO

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 99/11088	17/05/1999	16/05/1998

Applicant
THE PERKIN-EIMER CORPORATION et al.

1. **Where the International Searching Authority and the Receiving Office are not the same office:**

The applicant is hereby notified that the search copy of the international application was received by this International Searching Authority on the date indicated below.

Where the International Searching Authority and the Receiving Office are the same office:

The applicant is hereby notified that the search copy of the international application was received on the date indicated below.

21/06/1999 *(date of receipt).*

2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing in computer readable form.

3. **Time limit for establishment of international Search Report**

The applicant is informed that the time limit for establishing the International Search Report is 3 months from the date of receipt indicated above or 9 months from the priority date, whichever time limit expires later.

RECEIVED
ABD - LEGAL DEPT.
1999

@pls

4. A copy of this notification has been sent to the International Bureau and, where the first sentence of paragraph 1 applies, to the Receiving Office.

RECEIVED
AUG 2 1999

Name and mailing address of the International Searching Authority
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
ISA/EP

Form PCT/ISA/202 (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
BT-4584/PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 11088	17/05/1999	16/05/1998
Applicant		
THE PERKIN-EIMER CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/11088

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/25 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 736 333 A (MCBRIDE LINCOLN J ET AL) 7 April 1998 (1998-04-07) figures 1,2 ---	1-30
Y	EP 0 640 828 A (HOFFMANN LA ROCHE) 1 March 1995 (1995-03-01) figure 15 ---	1-30
A	WO 97 23649 A (BIORAD LAB INC) 3 July 1997 (1997-07-03) figure 1 ---	1-30
A	WO 97 46707 A (RASMUSSEN RANDY P ;UNIV UTAH RES FOUND (US); RIRIE KIRK M (US); WI) 11 December 1997 (1997-12-11) figure 11 --- -/--	1-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 1999	15/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mason, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/11088

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 622 455 A (BECTON DICKINSON CO) 2 November 1994 (1994-11-02) figures 1,5	1-30

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/11088

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5736333 A	07-04-1998	AU 3230297 A	05-01-1998
		CA 2256457 A	11-12-1997
		EP 0904411 A	31-03-1999
		WO 9746708 A	11-12-1997
EP 0640828 A	01-03-1995	AU 681682 B	04-09-1997
		AU 7141494 A	09-03-1995
		BR 9403338 A	11-04-1995
		CA 2129787 A	28-02-1995
		CN 1107892 A	06-09-1995
		CZ 9402078 A	15-11-1995
		FI 943936 A	28-02-1995
		HU 71622 A	29-01-1996
		IL 110732 A	15-07-1998
		JP 7163397 A	27-06-1995
		NO 943166 A	28-02-1995
		NZ 264310 A	25-03-1998
		PL 304805 A	06-03-1995
		SG 47865 A	17-04-1998
		ZA 9406330 A	28-02-1995
WO 9723649 A	03-07-1997	US 5792610 A	11-08-1998
		AU 1423597 A	17-07-1997
		CA 2241364 A	03-07-1997
		EP 0874912 A	04-11-1998
		EP 0879297 A	25-11-1998
		WO 9723648 A	03-07-1997
		US 5759781 A	02-06-1998
WO 9746707 A	11-12-1997	AU 3154797 A	05-01-1998
		AU 3380097 A	05-01-1998
		AU 3481297 A	05-01-1998
		CA 2256773 A	11-12-1997
		EP 0912760 A	06-05-1999
		EP 0906449 A	07-04-1999
		EP 0912766 A	06-05-1999
		WO 9746712 A	11-12-1997
		WO 9746714 A	11-12-1997
EP 0622455 A	02-11-1994	US 5371016 A	06-12-1994
		AU 668278 B	26-04-1996
		AU 5919894 A	27-10-1994
		BR 9401570 A	22-11-1994
		CA 2120993 A	27-10-1994
		JP 2738500 B	08-04-1998
		JP 6319524 A	22-11-1994

WO 99/60381
PCT/US99/11088

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
AKER, David
The Perkin-Elmer Corporation
850 Lincoln Centre Drive
Foster City, CA 94404
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 25 November 1999 (25.11.99)	
Applicant's or agent's file reference BT-4584/PCT 4529 WO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US99/11088	17 May 1999 (17.05.99)	16 May 1998 (16.05.98)

Applicant
THE PERKIN-ELMER CORPORATION et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   CA

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   25 November 1999 (25.11.99) under No. WO 99/60381

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

AKER, David
Perman & Green, LLP
425 Post Road
Fairfield, CT 06430-6232
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)
03 February 2000 (03.02.00)

Applicant's or agent's file reference
BT-4584/PCT

IMPORTANT NOTIFICATION

International application No.
PCT/US99/11088

International filing date (day/month/year)
17 May 1999 (17.05.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
AKER, David
The Perkin-Elmer Corporation
850 Lincoln Centre Drive
Foster City, CA 94404
United States of America

State of Nationality:
State of Residence:
Telephone No.: 203 761 5123
Facsimile No.: 203 761 2888
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
AKER, David
Perman & Green, LLP
425 Post Road
Fairfield, CT 06430-6232
United States of America

State of Nationality:
State of Residence:
Telephone No.: 203 259 1800
Facsimile No.: 203 255 5170
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Simin Baharlou

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

AKER, David
The Perkin-Elmer Corporation
761 Main Avenue
Norwalk, CT 06859-0199
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 21 July 1999 (21.07.99)	
Applicant's or agent's file reference BT-4584/PCT 4529 WO	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/11088	International filing date (day/month/year) 17 May 1999 (17.05.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 16 May 1998 (16.05.98)
Applicant THE PERKIN-ELMER CORPORATION et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
14 July 1998 (14.07.98)	60/092,784	US	19 July 1999 (19.07.99)

JUL 29 1999

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Marc Salzman
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

002744201

Form PCT/IB/304 (July 1998)

PCT/US99/11088

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

AKER, David
Perman & Green, LLP
425 Post Road
Fairfield, CT 06430-6232
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)	
03 February 2000 (03.02.00)	
Applicant's or agent's file reference BT-4584/PCT	**IMPORTANT INFORMATION**
International application No. PCT/US99/11088	**International filing date** (day/month/year) 17 May 1999 (17.05.99)
Priority date (day/month/year) 16 May 1998 (16.05.98)	
Applicant THE PERKIN-ELMER CORPORATION et al	

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,CA,CN,JP,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   None

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
Simin Baharlou

Telephone No. (41-22) 338.83.38